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Mode of Action of Datemycin on the Growth of Candida Albicans

I. Agglutination Reaction of Candida Albicans Observed in the Presence of Datemycin

By

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(Received for publication, April 11, 1959)

A little is known on the agglutination phenomena of any microorganisms with some antibiotics. Gros[1] was the first who reported in 1949 on the agglutination of several gram positive and also negative bacteria with streptomycin. Later on, in 1950, Lantterade and others[2] have noticed the agglutination of *Sta. albus* and *E. coli* with polymyxin.

Datemycin[3], an antibiotic possessing a limited inhibitory activity to a certain species of Candida have been isolated in this laboratory from a culture filtrate of a species of *B. subtilis*, M-14. When the crude substance of the antibiotic dissolved in phosphate buffer was shaken at 37°C with a suspension of *Candida albicans*, characteristic agglutination pattern have been found. Further studies on the site of action of datemycin in a slide cell culture system, revealed some interesting result suggesting the mechanism of the agglutination. In fact, mycelium formation of Candida albicans under the existence of this antibiotic was the real mode of this agglutination reaction. The result obtained along this line is the subject of this communication.

Experimental

Materials and Methods

Datemycin

Active principle in culture filtrate of *B. subtilis* (M-14) was once adsorbed to charcoal at pH 7.0 and eluted by 80% acetone at acidic pH. Further fractionation of the eluate was performed on the column of Al_2O_3. The active fraction was collected and dried in vacuo. Dried material was once dissolved in methanol and precipitated with acetone. Thus the crude datemycin was obtained. Further purification was performed by extracting the aqueous solution of datemycin with butanol containing 2

per cent *p*-toluensulfonic acid. From this butanol extract, the agent was again transferred to aqueous layer at pH 7.0. From this aqueous solution, *p*-toluensulfonic acid was removed by passing the fluid through the column of Amberlite IRA 411 (Cl type). After this procedure, purified preparation was obtained, though not crystallized. Crude datemycin was positive in both Fehling and ninhydrin test and had around the half activity of purified preparation. The latter was positive only in ninhydrin test and inhibited the growth of C. albicans in a concentration of 6 mcg/ml.

Candida

Eight strains of *Candida albicans*, i.e. M-2, M-8, M-9, M-10, Harvard, Takeda, FIA-1001, and Duke was used for this study. In addition, each one strain of *C. tropicalis*, *C. pseudotropicalis*, *C. krusei*, *C. parakrusei*, *C. guilliermondi* was also used. All these strains have been propagated on Sabouraud's medium at 37°C for 48 hours. Then they were collected by centrifugation, washed three times with phosphate buffer of pH 7.0 and suspended again in the same buffer. The concentration of the suspension was standardized turbidimetrically. Final concentration of these cells thus adjusted was 1.3×10^7/ml, corresponding to the wet weight of 2 mg/ml.

Agglutination test

Usually M-9 strain of *C. albicans* was used for this purpose, unless otherwise noted. Datemycin was dissolved in phosphate buffer of pH 7.0 and to 1 ml aliquots of this solution, equal amount of the cell suspension described above, was added. These tubes were usually shaken at 37°C for 4 hours and again left at room temperature until next morning. The result was read only macroscopically at the beginning of this study, but microscopical examination was also paralleled later. Usually the positive reaction became apparent after three hours of incubation but reached to the maximum after four hours. However, if these series of tubes were left overnight at room temperature, reading of the result was very easy. The concentration of the cell ranging from 0.5 mg to 2 mg did not affect this result seriously. The agglutination pattern here observed was rather flocculous than granular and only after repeated shaking of the tubes, homogenous turbidity was obtainable (Fig. 1).

Slide cell culture technique

According to the description of Nickerson,[4] slide cell culture of serveral Candidae was performed to test the site of action of this antibiotic. Basal medium contained 1.0 g of $(NH_4)_2SO_4$, 1 g of KH_2PO_4, 5.0 mcg of biotin, 15 g of agar and 20 g of soluble starch. They were dissolved in 1,000 ml of distilled water and 0.5 ml aliquot of the medium was spread on a slide glass in a circle of 1.5×2.0 cm^2. One loopful organisms taken from Sabouraud's slant was inoculated on the surface of this medium. The slide was closed by cover slips as usual and incubated at 27°C. After two

days of incubation, the mycelium formation and several changes which occurred in the cell was examined microscopically. In this medium, the excellent outgrowth of mycelium was followed with all of the strains tested, after 2 days of incubation. However, when 0.5 per cent of glucose or *M*/1,000 of cystein was added to this medium, the formation of mycelium was completely inhibited.

Results

Limited activity of this antibiotics on a species Candida albicans

As have been reported elsewhere[3], the antibiotic had an extremely limited range of activity, when the usual test organisms were examined for their susceptibility. One mg. of this antibiotic did not inhibit all tested bacteria both gram positive and negative. Neighter fungi were susceptible. However, when several species of Candida were tested, *C. albicans* was fairly susceptible to this antibiotic. One of the result obtained with purified preparation was shown in Table I. In this study, the activity

TABLE I

Effect of Datemycin on the Growth of Several Species of *Candida*

Candida tested	Minimal inhibition concentration (mcg/ml) obtained by	
	Agar streak dilution method*	Broth dilution method*
Candia albicans M-2	16	4
" " M-8	32	4
" " M-9	125	8
" " M-10	125	8
" " Harvard	16	8
C. krasei	500	63
C. parakrusei	>1000	125
C. tropicalis	>1000	250
C. stellatoidea	125	63

* Sabouraud's agar media and Sabouraud's broth were used.

was tested both by means of agar streak plate method and broth dilution method, where the activity was shown higher in the latter procedure. Thus all tested strains of *C. albicans* were susceptible. But the other species of Candida were not so susceptible as albicans. All of other species of Saccharomyces tested so far, were not inhibited with this antibiotic, though the result was omitted from the Table.

The specifity of the agglutination with regard to the tested organisms

The agglutinability of several strains described above was tested with this antibiotic. The preparation here used was a crude one. A typical result obtained from repeated experiments was illustrated in Table II.

TABLE II

Agglutination of Candida in the Presence of Datemycin

Candida tested	Concentration of datemycin (mcg/ml)									
	1000	500	250	125	63	32	16	8	4	0
C. albicans M-2	++*	++	++	+	−	−	−	−	−	−
M-8	++	++	++	+	−	−	−	−	−	−
M-9	++	++	++	+	−	−	−	−	−	−
M-10	++	++	++	+	−	−	−	−	−	−
Takeda	++	++	++	++	+	−	−	−	−	−
FIA 1001	++	++	++	+	−	−	−	−	−	−
Duke	++	++	+	±	−	−	−	−	−	−
Harvard	++	++	++	++	++	+	−	−	−	−
C. tropicalis	−	−	−	−	−	−	−	−	−	−
C. pseudotropicalis	−	−	−	−	−	−	−	−	−	−
C. krusei	−	−	−	−	−	−	−	−	−	−
C. parakursei	−	−	−	−	−	−	−	−	−	−
C. guilliermondi	−	−	−	−	−	−	−	−	−	−
C. stellatoidea	−	−	−	−	−	−	−	−	−	−

* Indicates the grade of agglutination.

All tested strains of *C. albicans* were agglutinated. But the other species of Candida which are known to be fairly resistant to this antibiotic as has been already shown in Table I, was not agglutinated at all. Thus the correlation was found between the agglutinability and sensitivity of the strain to this antibiotic. Several kind of gram negative and positive bacteria, which are already known to be resistant to this antibiotic were also tested for their agglutinability. They all gave the negative result.

One more remark to be noticed here is the fact, that when killed M-9 strain obtained after boiling for 10 minutes was tested for their agglutinability, the result was completely negative.

Optimum pH for this reaction was also tested with M-9. The result was shown in Table III and the highest titer was obtained at pH 7.0.

Optimum temperature for this reaction was also examined. When these tubes were left in an ice box all the way through after mixing the antibiotic with cell suspension, no agglutination patterns were found. The titer obtained at 37°C was not quite different from that obtained at 27°C.

TABLE III

Optimum pH for the Agglutination

pH	Concentration of datemycin (mcg/ml)							
	5000	2500	1250	630	320	160	80	0
4.0	##*	##	##	##	+	−	−	−
6.0	##	##	##	##	+	−	−	−
7.0	##	##	##	##	+	±	−	−
8.0	##	##	##	##	+	−	−	−
8.3	##	##	##	+	−	−	−	−

* Indicates the grade of agglutination.

Effect of sugars on the agglutination

On the final purification procedure of the antibiotic, the agglutination test was conducted in parallel with the inhibition test. Unexpected result here obtained was that the final preparation described in the section of materials and methods did not agglutinate M-9 at all. However, it was already known at that occasion that the final preparation of the antibiotic did not contaminated with Fehling positive substances, as has been pointed out before. Thus the participation of some sugars in this agglutination test have been suggested. In order to confirm the above idea, the agglutination reaction was examined with final preparation of datemycin but with and without glucose. As a result, apparent agglutination occurred only in the presence of glucose. Concentration effect of glucose was shown in Table IV.

As a result the concentration more than 0.01% was required for the stimulation of the reaction.

Other sugars than glucose were also tested for their effect on the agglutination. Among them, sucrose and galactose had the same activity as glucose, but only the weak reaction was obtained in the presence of mannose. Lactose, however, did not have this kind of activity at all (Table IV).

One has to notice here that *Candida albicans* are already known to ferment glucose, surcrose and galactose but not mannose and lactose, and the fact was also confirmed in my own hand. Thus the stimulation effect of sugars on the agglutination reaction was found only with sugars which are known to be fermented with this strain.

Microscopic examination of the agglutination reaction

The characteristics of the agglutination reaction described above will be summarized briefly as follows. Firstly, the reaction seems to be temperature dependent and the optimum pH for the reaction is 7.0. Secondly, only the susceptible cells to this antibiotic were agglutinated in the vegetative

TABLE IV

Effect of Sugars on the Agglutination of *Candida albicans* M-9 in the Presence of Purified Datemycin

Tested sugars	Concentration	Agglutination
Glucose	1 %	++*
"	0.1 %	++
"	0.01 %	++
"	0.001%	−
Mannose	1 %	±
Galactose	1 %	++
Lactose	1 %	−
Sucrose	1 %	++
Control		−

* Indicates the grade of agglutination.

state but not in killed form. Thirdly, only sugars which are utilized with these cells had the stimulating action on the agglutination. Thus the agglutination reaction here observed seems to be correlated rather to the growth of the tested strains than to be a known physical phenomenon.

For this reason, the agglutination pattern obtained in the test tubes were examined microscopically.

In the control tube, all of the cells were in yeast form after the incubation of 24 hours at 37°C. No characteristic change was found inside the cell. However, when 1 mg of datemycin was contained in the same system, the number of elongated cells first increased (See Fig. 1). This increase was detectable as early as two hours after the incubation. After 6 hours, macro-visible agglutination was found and the microscopic examination revealed that the cells are all in the form of mycelium. Thus the consistent change from yeast cell to elongated cell, and from elongated cell to the mycelium was established with all of the *C. albicans* tested in the presence of datemycin.

Mycelium formation in the slide cell culture

Mycelium formation described above was further examined in detail in the slide cell culture system.

The basal medium here used allowed the excellent mycelium formation of all the tested organisms but the addition of glucose in 0.5 per cent concentration inhibited the reaction completely. However, when datemycin was added in the medium in addition to 0.5% glucose, mycelium formation was again observed with Candida such as M-2, M-8 and M-9 (see Table V). At that time, the optimal concentration of datemycin to get the mycelium formation was between 2 to 8 mcg in the presence of

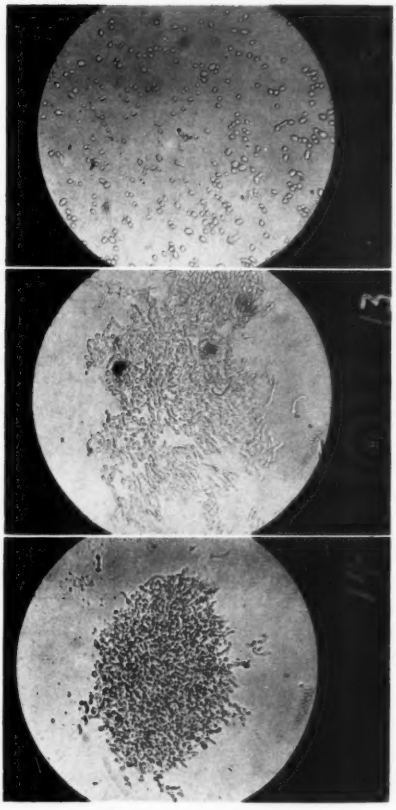

Fig. 1. Agglutination of *C. albicans* in the presence of datemycin.

0.5 per cent glucose. The higher concentration of the antibiotic, however, showed the toxic effect on the cell. The degeneration of the cell occurred first and mycelium formation did not follow.

When *C. tropicalis* and *C. krusei* which were known to be resistant to this antibiotic were used as test-organism, the mycelium formation was also found but only with higher concentration of the antibiotic such as 125 to 1,000 mcg. Within this concentrations, however, the degenerative change on the cell was not recognized.

In addition to glucose, the effect of cysteine on the mycelium formation was also tested. When M-8 strain was used for this purpose, though *M*/10,000 of cystein did not affect the formation of the mycelium, *M*/1,000 concentration inhibited the formation completely. Further, the effect of

TABLE V

Mycelium Formation Found in the Presence of Datemycin in the Medium Containing 0.5% Glucose

Concentration of datemycin (mcg/ml)	Tested candidae											
	M-2		M-8		M-9		Takeda		C. trop		C. krusei	
	m*	D†	m	D	m	D	m	D	m	D	m	D
1000	–	(卅)	–	(卅)	–	(卅)	–	(卅)	卅		卄	
125	–	(卅)	–	(卅)	–	(卅)	–	(卅)	卅		+	
32	–	(卄)	–	(卄)	卅		–	(+)	–	(–)	–	(–)
8	卅		卅		卅		卅		–	(–)	–	(–)
2	卄		–	(–)	–	(–)	–	(–)	–	(–)	–	(–)
0	–	(–)	–	(–)	–	(–)	–	(–)	–	(–)	–	(–)

* Grade of mycelium formation. † Grade of degeneration of cells.

TABLE VI

Effect of Datemycin on the Growth of *C. albicans* M-8 in the Medium Containing Different Concentration of Cysteine

Concentration of datemycin (mcg/ml)	Concentration of cysteine													
	1/100 *M*		1/300 *M*		1/500 *M*		1/800 *M*		1/1,000 *M*		1/10,000 *M*		0	
	m*	D†	m	D	m	D	m	D	m	D	m	D	m	D
1000	–	(–)	–	(–)	–	(+)	–	(卄)	–	(卅)	–	(卅)	–	(卅)
500	–	(–)	–	(–)	–	(–)	–	(卄)	–	(卅)	–	(卅)	–	(卅)
250	–	(–)	–	(–)	–	(–)	–	(卄)	–	(卅)	–	(卅)	–	(卅)
125	–	(–)	–	(–)	–	(–)	–	(卄)	–	(卅)	–	(卅)	–	(卅)
63	–	(–)	–	(–)	–	(–)	–	(+)	–	(卅)	–	(卅)	–	(卅)
32	–	(–)	–	(–)	–	(–)	–	(+)	–	(卅)	–	(卅)	–	(卅)
16	–	(–)	–	(–)	–	(–)	–	(–)	卄		–	(卅)	–	(卅)
8	–	(–)	–	(–)	–	(–)	–	(–)	卅		卅		–	(卅)
4	–	(–)	–	(–)	–	(–)	–	(–)	卅		卅		–	(卄)
2	–	(–)	–	(–)	–	(–)	–	(–)	–	(–)	卅		卅	
1	–	(–)	–	(–)	–	(–)	–	(–)	–	(–)	卅		卅	
0.5	–	(–)	–	(–)	–	(–)	–	(–)	–	(–)	卅		卅	
0	–	(–)	–	(–)	–	(–)	–	(–)	–	(–)	卅		卅	

* Grade of mycelium formation. † Grade of degeneration of cells.

datemycin on the cell growing in the presence of different concentration of cysteine was examined (see, Table VI). The formation of mycelium with M-8 was inhibited with *M*/1,000 cysteine, but the addition of datemycin ranging from 4 to 8 mcg allowed the formation of mycelium. The higher

concentration of datemycin, such as 32 mcg again induced the degeneration of the cells. This degenerative effect was found with 4 mcg concentration of the antibiotic without addition of cysteine. Thus the interesting result here obtained, is the competitive effect of cysteine on the activity of datemycin. For example, when the concentration of cystein was shifted to *M*/500, only slight degeneration was obtained at the concentration as high as 1 mg per ml of datemycin but not at the concentration of 32 mcg. If the concentration of cystein was *M*/300, even 1 mg of datemycin did not affect the state of cell at all. On the basis of this observation, the competitative effect of cystein on the inhibitory activity of datemycin was tested later. In the presence of *M*/100 of cystein, the concentration of datemycin inhibiting the growth of M-9, dropped from 2 mcg to 50 mcg.

For comparative study, *C. kursei*, known as resistent to this antibiotic was also tested in the same manner (see Table VII). In the presence of

TABLE VII

Effect of Datemycin on the Growth of *C. krusei* in the Medium Containing Different Concentration of Cysteine

Concentration of datemycin (mcg/ml)	Concentration of cysteine									
	1/100 *M*		1/500 *M*		1/1000 *M*		1/10000 *M*		D	
	m*	D†	m	D	m	D	m	D	m	D
1000	–	(–)	###		###		###		###	
500	–	(–)	###		###		###		###	
250	–	(–)	###		###		###		###	
125	–	(–)	+	(–)	###		###		###	
63	–	(–)	–	(–)	##		###		###	
32	–	(–)	–	(–)	##		###		###	
16	–	(–)	–	(–)	##		###		###	
8	–	(–)	–	(–)	+		###		###	
4	–	(–)	–	(–)	–	(–)	###		###	
2	–	(–)	–	(–)	–	(–)	###		###	
1	–	(–)	–	(–)	–	(–)	###		###	
0.5	–	(–)	–	(–)	–	(–)	###		###	
0	–	(–)	–	(–)	–	(–)	###		###	

* m grade of mycelium formation. † D grade of degeneration of cells.

M/1,000 of cysteine, 8 mcg or more concentration of datemycin allowed the mycelium formation. In increasing the concentration of cysteine, mycelium formation was found only in the presence of high concentration of datemycin. Thus the activity of stimulating the mycelium formation was shown also with this fairly resistant strain, but in a little bit higher

concentration.

As far as the degenerative effect is concerned, however, even in the absence of cysteine, no effect was found on *C. kursei* with 1 mg of datemycin. This was quite different from the result obtained with susceptible strains.

Discussion

As has been pointed out in the preface of this communication, two papers[1)2)] have already appeared on the agglutination reaction of bacteria caused by the addition of antibiotics. Two antibiotics, streptomycin and polymyxin, which has the basic character in nature reacted with the RNA on the surface of gram-positive bacteria and formed an insoluble complex. Thus the agglutination phenomena have been elucidated in these cases.

In this case, however, the site of agglutination was quite different. It was the mycelium formation of Candida by the addition of an antibiotic, datemycin. The optimum pH of the reaction was 7.0 and the reaction required the temperature. Only susceptible cells of Candida were agglutinated with lower concentration of the antibiotic. But these cells have to be in an alive state, because heat killed organisms were not agglutinated. The addition of some sugars, such as glucose, which were fermented by this organism were necessary for this agglutination. The addition of cystein, which is known to inhibit the formation of mycelium[5)6)] inhibited this agglutination. Thus all of the circumstantial evidence suggest that the mycelium formation is a site of this agglutination and the idea was confirmed by microscopic examination as has been illustrated in Fig. 1.

The action of datemycin was reversed by cysteine both in the inhibition test and in the mycelium formation as was the case with penicillin and streptomycin. Stimulative activity of the antibiotic in the formation of mycelium will be correlated to this fact, if one assume that the inhibition of the SH-protein synthesis is correlated to the formation of elongated cell[5)7)8)]. The experimental result which was not described here indicates that the sensitive strain to this antibiotic contains lower concentration of SH-compound when compared to that of resistant strain.

In spite of the fact that cysteine reversed the action of this antibiotic, the higher inhibitory activity of datemycin was obtained in a fluid culture system rather than in an aerobic condition, such as on the surface of the agar. This observation holds true if one compare the concentration of datemycin, which cause the agglutination in test tubes and the concentration which cause the mycelium formation in a closed slide cell culture system. In the former test at least 25 mcg was necessary to cause the agglutination of M-8 but in the latter test as low as 4 mcg of the antibiotic stimulated the mycelium formation. Rather the concentration of the

organisms tested in these systems or the nutritional condition of the medium should be taken into account for these discrepancies.

Summary

In the presence of an antibiotic, datemycin, the agglutination phenomenon of *C. albicans* was first recognized. Further study revealed that the agglutination reaction was restricted to only susceptible strains, and these cells have to be in vegetative state but not killed. The reaction was evident only in room or blood temperature but not in the cold. The optimum pH was in the vicinities of 7.0. Sugars which are utilized by the tested organisms had the stimulating effect on the agglutination. Further microscopic examination revealed that the mycelium formation of the tested cells was the real mode of this agglutination.

In a slide cell culture system, the fact that the lower concentration of datemycin stimulates the mycelium formation of Candida was quite evident. Both agglutinative and inhibitory activities of datemycin was partly reversed by the addition of cysteine.

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Studies on Cerebral Circulation and Metabolism in Hypertensive Diseases

I. The Influence of Aging on Cerebral Circulation and Metabolism

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(Received for publication, March 30, 1959)

Most of the studies on the cerebral circulation and metabolism in essential hypertension using N_2O method have been made mainly in patients of less than 50 years in age. While Shenkin[2] denied the influence of aging on the cerebral circulation and metabolism, which was first pointed out by Fazekas[1], more recently, a series of authors[3 4 5] have affirmed some changes in it with the rise in age. Fazekas[6] and Tazaki[7] investigated the cerebral circulation and metablism in essential hypertension in consideration of difference in age, but the results obtained by them were not always in agreement and some points are yet left in doubt. Since just these discrepant findings concern the parts of the problem most important for the pathophysiological interpretation of the cerebral circulation and metabolism in essential hypertension, the author has undertaken the present investigation, following up in healthy normotensive persons and in subjects with essential hypertension the difference of the effects of aging on the cerebral circulation and metabolism and its pathophysiological singnificance.

Experimental

Method

The subjects consisted in 24 normotensive persons aged between 20 and 76 years without evidence of cardiac, renal or cerebrovascular disease and in 51 cases with essential hypertension between 30 and 76 years of age. The cerebral blood flow was measured with the method of Kety and Schmidt[8] and the blood N_2O content with Van Slyke and Neill's method[9]. The blood oxygen content has been calculated from the blood oxygen capacity and the blood oxygen saturation by the following formula;

$$\text{Blood oxygen content (Vol \%)} = \frac{\text{Blood oxygen capacity (Vol \%)} \times \text{Blood oxygen saturation (\%)}}{100}.$$

In this formula, the blood oxygen capacity was measured by Shigiya's method[10] and the blood oxygen saturation by Tokita's method[11], respectively using spectrophotometer. Arterial blood pressure was measured by the auscultatory method with the arm at heart level. Units concerning the cerebral circulation and metabolism are listed in the following:

Mean arterial blood pressure: MABP (mm. Hg) = diastolic pressure $+\frac{1}{3}$ pulse pressure.

Cerebral blood flow: CBF (cc./100 g. brain/min.)

Cerebral vascular resistance: CVR (mm.Hg/cc.blood/100 g. brain/min.) = MABP/CBP.

Cerebral arteriovenous oxygen difference: (A–V) O_2 (Vol %)

Cerebral oxygen consumption ($CMRO_2$ (cc./100 g. brain/min.) = CBF $\times \frac{\text{(A–V) } O_2}{100}$

Results

1. *The effect of aging in normotensive persons*

The relation between the individual values for cerebral circulation and metabolism and their ages in 24 normotensive persons ranging from 20 to 76 years of age is shown in Fig. 1. CBF showed rather high values

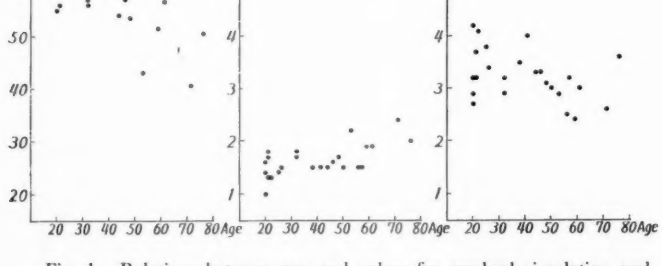

Fig. 1. Relations between age and values for cerebral circulation and metabolism in normotensive subjects.

in the subjects between 20 and 29 years, tending to decrease slowly between 30 and 49 years and somewhat more apparently in the ages above 50. CVR tended to increase slowly with age, while $CMRO_2$ remained unchanged between 20 and 49 years but slowly declined beyond 50 years. When the subjects were divided into the groups of young (20–29 years), middle (30–49 years) and old (above 50 years) ages, the mean values of each groups were as shown in Table I. In the middle-age group, the

TABLE I

Mean Values for Cerebral Circulation and Metabolism in Young, Middle and Old Age Groups of Normotensive Subjects

	Cases	Age range	Mean age	MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$
Young age group	9	20-26	22	91	63.9	1.44	5.42	3.46
Middle age group	7	32-48	40	93	57.1	1.63	5.83	3.32
Old age gronp	8	50-76	60	97	53.9	1.85	5.48	2.90

mean CBF was slightly lower, the mean CVR and $(A-V)O_2$ slightly higher, than in the young age group, while mean $CMRO_2$ showed no change. In the old age group, the mean CVR was found slightly risen, but the mean CBF, $(A-V)O_2$ and $CMRO_2$ a little lowered, as compred with those of the middle-age group. Thus, in the old age group, the CBF and $CMRO_2$ were found lower but th mean CVR higher than in the other younger groups.

2. *The effect of aging in cases with essential hypertension*

In Fig. 2 are plotted individual values for cerebral circulation and metabolism in 51 patients with essential hypertension according to each age ranging from 30 to 69 years of age. CBF and $CMRO_2$ here showed a comparatively steep tendency downwards with age while CVR increased steeply too, these tendencies being particularly obvious in the age over 50 years.

The mean values in the two groups of middle (30–49 years) and old (above 50 years) ages are tabulated in Table II. In the old age group, the mean CBF and $CMRO_2$ were lower, the mean CVR higher than in the middle age group, but the mean MABP and $(A-V)O_2$ showed no apparent change by age.

3. *Comparison of the effects of aging between the normotensive subjects and the cases with essential hypertension of the same age range*

In Table III A are tabulated the mean values for the cerebral circulation and metabolism of normotensive group and hypertensive group of

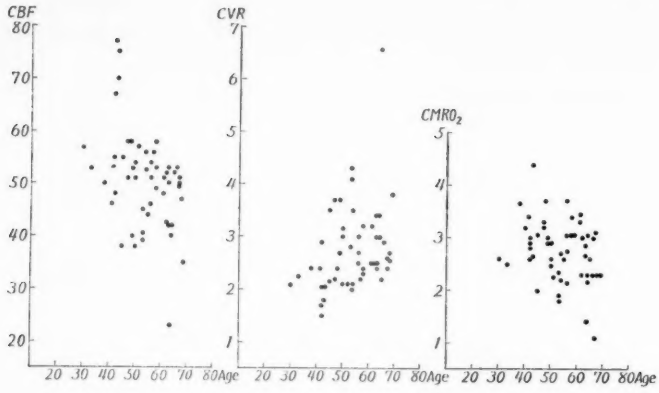

Fig. 2. Relations between age and values for cerebral circulation and metabolism in patients with essential hypertension.

TABLE II

Mean Values for Cerebral Circulation and Metabolism in Middle and Old Age Groups of Patients with Essential Hypertension

	Cases	Age range	Mean age	MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$
Middle age group	17	30–49	43	131	56.0	2.44	5.58	3.05
Old age group	34	50–69	59	134	47.9	2.90	5.55	2.62

middle age in comparison. In the hypertensive group, the mean MABP and CVR were found markedly higher than in the normotensive group, while the mean CBF, $CMRO_2$ and $(A-V)O_2$ showed no significant difference by groups.

In Table III B are shown the same values in old age groups. Here, in the hypertensive group, besides the marked increase in the mean MABP

TABLE III A

Mean Values for Cerebral Circulation and Metabolism in Normotensive Group and Hypertensive Group in the Middle Age

	Cases	Age range	Mean age	MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$
Normotensive group	7	32–48	40	93	57.1	1.63	5.83	3.32
Hypertensive group	17	30–49	43	131	56.0	2.44	5.58	3.05

TABLE III B

Mean Values for Cerebral Circulation and Metabolism in Normotensive Group and Hypertensive Group in the Old Age

	Cases	Age range	Mean age	MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$
Normotensive group	8	50-76	60	97	53.9	1.85	5.48	2.90
Hypertensive group	34	50-69	59	134	47.9	2.90	5.55	2.62

and CVR, the mean CBF and $CMRO_2$ showed a slight decrease, while in the mean $(A-V)O_2$ was seen no appreciable difference between the groups.

4. *Comparison of the values for cerebral circulation and metabolism between normotensive subjects and cases with essential hypertension, with and without sclerosis of retinal arterioles in the same age range*

The cases with essential hypertension were divided into the two groups of those with and without sclerosis in their retinal arterioles and the mean values for cerebral circulation and metablism of these two groups and of a group of normotensive subjects of the same age range were studied in comparison.

1) Comparison in middle age groups

In Table IV A the mean values of each group of the middle age are compared. In the hypertensive group without sclerosis of retinal arterioles,

TABLE IV A

Mean Values for Cerebral Circulation and Metabolism in Normotensive Group and Hypertensive Group, with and without Sclerosis of Retinal Arterioles in the Middle Age

	Cases	Age range	Mean age	MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$
Normotensive group	7	32-46	40	93	57.1	1.63	5.83	3.32
Hypertensive group without retinal arteriolosclerosis	8	33-49	41	122	57.1	2.19	5.56	3.10
Hypertensive group with retinal arteriolosclerosis	9	30-49	44	140	55.1	2.66	5.60	3.05

the mean MABP and CVR were markedly higher than those of the normotensive group, but the mean CBF, $(A-V)O_2$ and $CMRO_2$ showed no apparent difference from the normotensive group. In the hypertensive

TABLE VI B

Mean Values for Cerebral Circulation and Metabolism in Normotensive Group and Hypertensive Group, with and without Sclerosis of Retinal Arterioles in the Old Age

	Cases	Age range	Mean age	MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$
Normotensive group	8	50–76	60	97	53.9	1.85	5.48	2.90
Hypertensive group without retinal arteriolosclerosis	3	62–66	64	133	52.5	2.53	5.55	2.88
Hypertensive group with retinal arteriolosclerosis	31	51–69	60	135	47.5	2.94	5.56	2.59

group with sclerosis of retinal arterioles, the mean MABP and CVR were found augmented prominently over those in the normotensive group and perceptibly above those in the hypertensive group without it. But the mean CBF and $CMRO_2$ were found slightly but not significantly lower than those in the other two groups.

2) Comparison in old age groups

In Table IV B are shown the same values in the old age groups in comparison. In the hypertensive group without sclerosis of retinal arterioles, the mean MABP and CVR were found significantly increased over those in the normotensive group, but there was noted no significant difference in the mean CBF, $(A-V)O_2$ and $CMRO_2$. In the hypertensive group with sclerosis of retinal arterioles the mean MABP and CVR were even more remarkably augmented, and besides, the mean $(A-V)O_2$ was not different and the mean CBF and $CMRO_2$ were slightly lowered from those in the normotensive group. As compared with the values in the hypertensive group without sclerosis of retinal arterioles the mean CVR showed marked increase and the mean CBF and $CMRO_2$ slight decrease, while the mean MABP and $(A-V)O_2$ were found not different.

DISCUSSION

1. On normotensive cases

As stated above, with advance in age, the CBF and $CMRO_2$ slowly declined while CVR gradually increased, these tendencies being more marked in the subjects above 50 years in age. The results obtained by Fazekas[6], Scheinberg[3] and Heyman[4] were similar, indicating increase of CVR and decrease of CBF and $CMRO_2$ beyond about 50 years of age. Aizawa[5] reported results similar to those as far as CBF and CVR are concerned, but he denied the decrease of $CMRO_2$ in higher age. At any

rate, it seems justifiable to interprete such changes in the cerebral hemodynamics in advanced age as due to the progress of cerebral arteriosclerosis even in normotensives with age, as Fazekas[6] and Scheinberg[3] have suggested. In my present studies it has been noted, that in the middle age group the mean CBF is lower than in the young one, but owing to the increase in the mean $(A-V)O_2$ no change by age is observed in the mean $CMRO_2$, while in the old age group the mean CBF is lowered and at the same time the mean $(A-V)O_2$ remains unchanged or decreases, as compared with values in the other two groups, so that the mean $CMRO_2$ has also been forced down. Such a difference in findings is hardly interpretable by citing sclerosis of the cerebral arteries. Scheinberg[3] obtained similar results in his study and interpreted them as due to the lowered function of the cerebral cells accompanied by the lowering of CBF. This hypofunction of the cerebral cells is probably caused, besides by the long-standing lowering of CBF, also by an essential senility process.

2. On cases with essential hypertension

In the cases with essential hypertension, the influence of aging upon the cerebral circulation and metabolism is more apparent than in normotensive subjects. In this respect, the results obtained by Fazekas[6] and Tazaki[7] are similar to those of the present studies, while Kato[12] denies any change by age of $CMRO_2$ in them. In order to elucidate the mechanism of such changes the difference of the influences of aging between the normotensive and the hypertensive group in the present investigation has been closely examined and found that in the middle age, only the mean MABP and CVR are markedly increased in the hypertensive group above those in the normotensive group. This is in good agreement with the results obtained by other investigators[2][13][14] and it seems to suggest that before 50 years of age, little change is occasioned in the cerebral circulation and metabolism, in spite of the increase in CVR. On the other hand, in the hypertensive group of the old age, as shown above, besides the marked increase in MABP and CVR, a slight decrease in CBF and $CMRO_2$ are observed. Tazaki[7] also reports on the tendency towards decrease of CBF and $CMRO_2$ in such hypertensive patients of more than 50 years of age below the values in normotensive subjects of the same age range. On the other hand, Fazekas[6] denies any change in CBF and $CMRO_2$, but in his cases, there is a wide difference in the mean age between his hypertensive group and normotensive group, and it is hard to justify the interpretation he made by such data alone.

One of the causes of the decrease of CBF in essential hypertension is accepted as lying in the marked increase of CVR, and these changes with aging, observed more markedly in the hypertensive cases than in the normotensive subjects, are most likely due to the coexistance of cerebral

arteriosclerosis accompanied by the state of hypertension.

In order to obtain further information on this point, the author has investigated with the hypertensive cases divided into the groups of those without and those with retinal arteriolosclerosis and found, that in the middle age, the mean MABP and CVR are markedly increased in the former group above those in the normotensive group, but that CBF and $CMRO_2$ show no appreciable difference, while in the latter group with retinal arteriolosclerosis MABP and CVR are even more markedly increased, but the mean CBF and $CMRO_2$ being not significantly different as compared with those in the normotensive group. In the old age group too, MABP and CVR are found markedly increased in the hypertensive group without retinal arteriolosclerosis above those in the normotensive group, CBF and $CMRO_2$ remaining not different, while in the hypertensive group with retinal arteriolosclerosis, besides the even more marked increase in the mean MABP and CVR, a slight decrease in CBF and $CMRO_2$ is observed below those in the normotensive group. Considering that in those hypertensive cases, the retinal arteriolosclerosis are generally more advanced in the old patients than in the middle age patients, the above results seem to suggest that the cerebral arteriolosclerosis is more expedited with age in patients with essential hypertension than in the normotensive subjects and the manifestation of severe disturbances in the cerebral circulation and metabolism in the old age seems to enhance the significance of cerebral arteriolosclerosis on the cerebral circulation and metablism in essential hypertension.

Summary

The values concerning the cerebral circulation and metablism of 24 healthy normotensive subjects and 51 patients with essential hypertension were measured, for studying the influence of aging on those functions, and also the significance of the complication of cerebral arteriolosclerosis, so closely related with aging, was discussed.

1. In any group of normotensive as well as of hypertensive subjects, cerebral vascular resistance (CVR) tended to increase and cerebral blood flow (CBF) and cerebral oxygen consumption ($CMRO_2$) to decrease with advance in age. This tendency was more evident in the latter than in the former group.

2. The mean values concerning the cerebral circulation and metabolism of the patients with essential hypertension were studied in comparison with those of normotensive subjects, and it was found that the mean arterial blood pressure (MABP) and CVR were found markedly higher in the former, whether of the middle or old age, than those of the latter subjects of the same age range. The mean CBF and $CMRO_2$ were not

found significantly different in the normotensive and the hypertensive subjects of the middle age groups, but in the old age groups those values were slightly lowered in the subjects with essential hypertension.

3. When the patients with essential hypertension are complicated with retinal arteriolosclerosis, in general the increase in CVR was more prominent, in particular, more so in old patients, and simultaneously, in not a few cases, the disturbance in cerebral circulation and metabolism was more evident, CBF and $CMRO_2$ showing a tendency of lowering.

The expense of this study was in part defrayed by a Grant in Aid for Medical Research, Welfare Ministry. The author wishes to express his thanks to Dr. S. Sato, chief doctor of our Medical Clinic for co-operation in carrying out this investigation.

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Studies on Cerebral Circulation and Metabolism in Hypertensive Diseases

II. Relation between the Cerebral Circulation and Metabolism and Clinical Findings in Essential Hypertension

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It is rationally suggested that in hypertension the blood circulation and intermediate metablism in the brain undergo the influence of the presence of various clinical findings and complications of the disease and vice versa. Many problems, however, concerning those relations are not yet adequately elucidated. In the present report, these phases have been strongly studied and the correlation between the values representing the cerebral circulation and metablism and the clinical findings on 51 patients with essential hypertension and its pathophysiological meaning have been discussed.

Experimental

Method

As subjects, 51 patients with essential hypertension were used and the method of measurement of the cerebral circulation and metabolism is the same as given in the first report[1]. 35 of the cases were examined on their arm-to-ear circulation time by Matsuoka's method[2]*, 11 on their renal hemodynamic data [renal blood flow (RBF) and renal vascular resistance (RVR)] by sodium para-aminohippurate clearance method, 28 on renal function with 15 min. phenolsulphonphthalein excretion test and all the subjects on the haemoglobin content of the arterial blood with Shigiya's method[3].

Results and Discussion

1. *Retinal findings and cerebral circulation and metabolism*

* Acetosulfonamide solution is given in cubital vein and blood blots are obtained from ear lob every one second. The first point where acetosulfonamide appears in blot is measured by using aldehyd reagent.

It is generally accepted that the retinal findings and the prognosis of essential hypertension are closely correlated; the present author has also suggested in the study reported previously[1] that apparent disturbances in the circulation and metablism of the brain are found in the cases with severe sclerosis of the retinal arterioles. In Fig. 1 are shown the individual

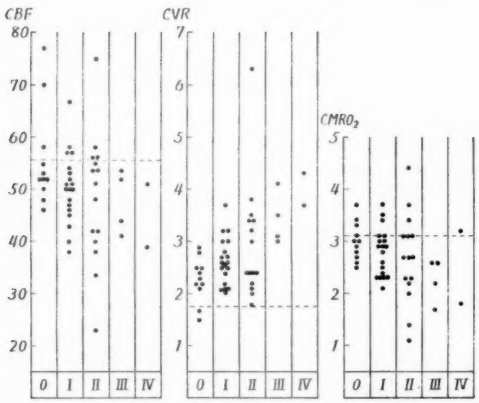

Fig. 1. Relations between retinal findings according to Keith-Wagener's classification and cerebral circulation and metabolism.
0–IV: Keith-Wagener's grade of retinal findings.
---: Mean values in normotensive subjects.

values concerning the cerebral circulation and metablism in the patients of the essential hypertension divided into four groups according to the Keith-Wagener's classification of the retinal changes. With the progress of the retinal findings from the group of O grade (no change) to IV grade group, CBF and $CMRO_2$ tended to lower and CVR to rise, and in paticular, CVR was found in closest parallelism with the retinal changes. The same fact is reported also by Aizawa[4] and Kato[5]. Leopold *et al.*[6] who adopted a finer gradation of the retinal findings based on Keith-Wagener's classification principle, have found that CVR changes in parallel with those finer gradation too, while Hafkenshiel[7] and Bernsmeier[8] denied such a correlation. The present results seem to suggest a close correlation between the retinal changes and the organic vascular changes in the brain.

2. *Cardiovascular findings and cerebral circulation and metaboliem*

Essential hypertension is often accompanied by cardiac disturbances. Frequently in the cases, in which no notable anatomical changes in the brain could be observed, but succumbed to apoplectic strokes, the existance of cardiac factors has been presumed[9]. So, it must be of importance from clinical point of view to study the changes in the circulatory and metabolic

functions of the brain in essential hypertension with the cardiac disturbances, which repeatedly occur as complication. For this purpose, the author examined in subjects with essential hypertension the possible relations between the heart roentgenographic and the electrocardiographic findings and the circulation time on the one hand, and the values concerning the cerebral circulation and metabolism on the other.

First, the subjects were divided into the two groups of those showing and those free of left ventricular hypertrophy on their X-ray findings. Their individual values concerning the cerebral circulation and metabolism are shown in comparison by groups in Fig. 2. In the group with the hypertrophy, the frequency of the cases with lowered CBF and $CMRO_2$ and increased CVR was higher than in the hypertrophy-free group. Fig. 3 shows in comparison the values in the group of the subjects showing pre-

Fig. 2

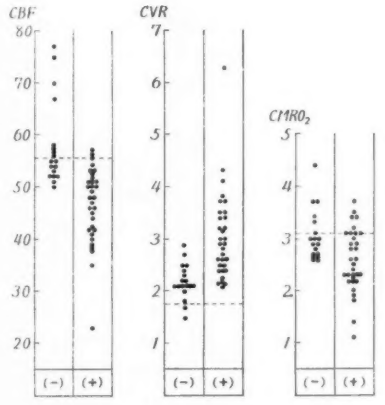

Fig. 3

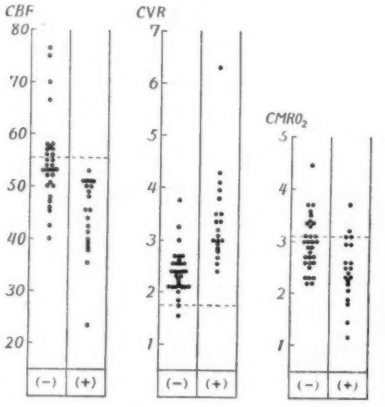

Fig. 2. Relations between X-ray findings in heart and cerebral circulation and metabolism.
Group (−): Cases without left ventricular hypertrophy.
Group (+): Cases with left ventricular hypertophy.
---: Mean values in normotensive subjects.

Fig. 3. Relations between electrocardiographic findings and cerebral circulation and metabolism.
Group (−): Cases without ECG findings such as myocardial damage, coronary insufficiency or left ventricular hypertrophy.
Group (+): Cases with those findings.
---: Means values in normotensive subjects.

sence of left ventricular hypertrophy, coronary insufficiency or myocardial damage and of those free of such findings, as diagnosed electrocardiographically. In the former group, CBF and $CMRO_2$ were lowered and CVR

elevated in a larger number of the cases than in the latter.

In Fig. 4 is shown the relation of the arm-to-ear circulation time to CBF and CVR. A slight tendency of lower CBF and higher CVR was found in the cases with prolonged circulation time.

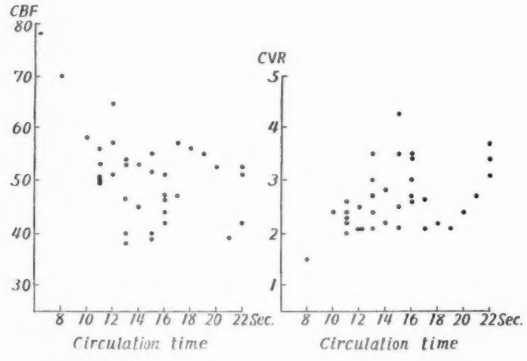

Fig. 4. Relations between arm-to-ear circulation time and cerebral circulation.

Yokomatsu[10] has found higher CVR and lower CBF and $CMRO_2$ in the hypertensive group with myocardial damage in complication than in the group free from the complication; also Kato[5] states that among cases with lowered CBF cardiac hypertrophy and myocardial damage are frequently found. Those findings are in agreement with the present author's cited above. Thus, it is obvious that in essential hypertension the cerebral circulatory and metablic changes are closely correlated with the cardiac disturbances; but it cannot be decided off-hand whether those changes in the brain are caused merely by the reduction of cardiac function or by other factors coexisting in such cases. This question requires further studies.

3. *Renal function and cerebral circulation*

Disturbances in renal functions are also frequently found in essential hypertension and some relation between the renal functions and the circulatory changes in the brain seems to be presumable. In the present studies the relation between the hemodynamics and dye excretion function of the kindey on the one hand and the cerebral circulation on the other has been investigated. In Fig. 5 are shown the relation between CBF and RBF and that between CVR and RVR. In some instances with lowered RBF, CBF is also found lowered, but such cases are small in number and no close correlation can be established between these two values. Between RVR and CVR a slight parallelism is confirmed.

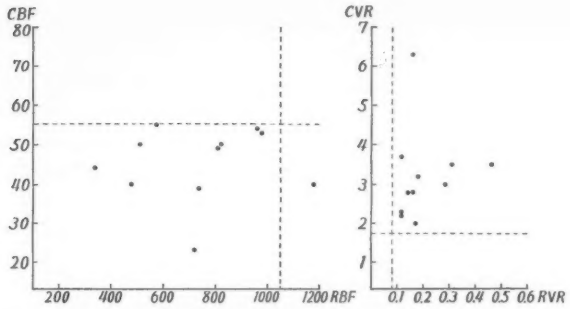

Fig. 5. Relations between renal hemodynamics and cerebral circulation.
RBF: Renal blood flow. RVR: Renal vascular resistance.
---: Mean values in normotensive subjects.

Fig. 6 demonstrates the relations of P.S.P. excretion value from the kidney in 15 minutes versus CBF and CVR. In the cases with lowered *P.S.P.* excretion there are tendencies of lowered CBF and elevated CVR, a parallelism to some extent being established between *P.S.P.* excretion value and the other two values.

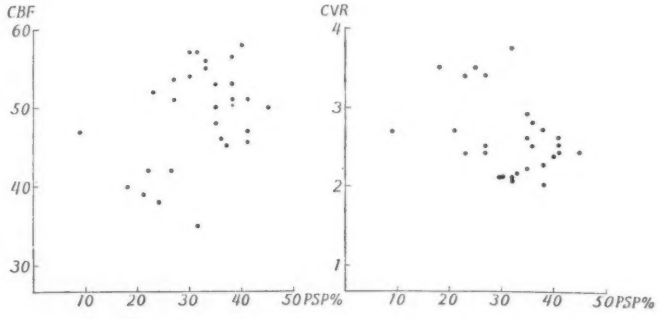

Fig. 6. Relations between phenolsulphonphthalein excretion value in 15 min. and cerebral circulation.

Aizawa[11] has found a significant positive correlation between CBF and RBF in essential hypertension; Atarashi[12] also states that in older patients of essential hypertension there exists an apparent correlation between CBF and RBF, both these values dropping abreast, while Kato[5] insists that RBF tends to be lowered the further, the lower the CBF and the $CMRO_2$, but that no definite correlation can be established between these values.

From the data of the present investigation, it may be suggested that there is a correlation to some extent between the renal function and the cerebral circulation, while no such close parallelism as found between the retinal and cardiac findings and the cerebral circulation and metabolism has been confirmed here. But, as shown above, the fact that the cerebral circulation and metabolism are generally more severely disturbed in the cases of essential hypertension with retinal changes, cardiac or renal disturbances than in those free of such complications is worthy of clinical attention.

4. *Anemia and cerebral circulation and metabolism*

According to Heyman and others[13] in cases with chronic anemia CBF is increased, but no such a fact has been reported as observed among the patients with essential hypertension. In the present study it has been observed that some tendency of increased CBF may be found among the patients of essential hypertension with lowered hemoglobin content, as seen in Fig. 7, where relation between the arterial hemoglobin content and CBF is graphically shown.

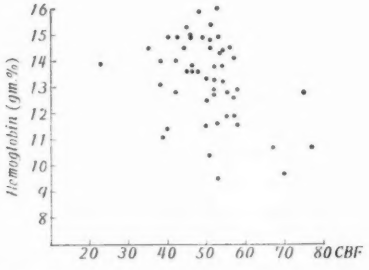

Fig. 7. Relation between hemoglobin content in arterial blood and CBF.

This seems to represent a kind of homeostasis against the hazard of oxygen deficiency in the brain due to anemia. Thus, anemia too seems to require notice as an important factor changing CBF in essential hypertension.

Summary

On 51 cases of essential hypertension the relations between the clinical findings and the cerebral circulation and metabolism measured by N_2O method have been investigated and the following facts elucidated:

1. With the advance of the retinal findings according to Keith-Wagener's classification, cerebral blood flow (CBF) and cerebral oxygen consumption ($CMRO_2$) tend further to decrease and cerebral vascular resistance (CVR) to increase. The latter has a particularly close paralle-

lism with the retinal findings.

2. In the group showing the hypertrophy of the left ventricle on the roentgenogram or the group, in which the left ventricular hypertrophy, coronary insufficiency or myocardial damage is suggested by the electrocardiographical findings, the disturbances in the cerebral circulation and metabolism are found the severer than in the groups without such finding. Similar relation in the group with prolonged circulation time may be confirmed.

3. No close parallelism is established between CBF and renal blood flow (RBF), while some correlation is found between CVR and renal vascular resistance (RVR), also, between the renal function, as tested with P.S.P. excretion, on the one hand and CBF and CVR on the other.

4. In the cases with lowered hemoglobin content of the blood, CBF is to some extent augmented.

The expense of this study was in part defrayed by a Grant in Aid for Medical Research, Welfare Ministry. The author wishes to express his thanks to Dr. S. Sato, chief doctor of our Medical Clinic for co-operation in carrying out this investigation.

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Studies on Cerebral Circulation and Metabolism in Hypertensive Diseases

III. The Effect of Prolonged Oral Administration of Hypotensive Agents upon the Cerebral Circulation and Metabolism in Patients with Essential Hypertension

By

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A series of investigations have been reported on the significance of hypotensive agents in restraining aggravation of the hypertension, preventing the progression of the organic vascular changes and minimizing complications. The studies on cerebral circulation and metabolism under lowered blood pressure induced by those agents is a theme of importance, but on this subject mainly the acute effect of lowering blood pressure by those agents has been investigated, and reports on the effect of prolonged oral administration, the mode of application clinically most important, are few.

From this view-point, the author undertook a study on the changes of the cerebral circulation and metabolism following prolonged oral administration of the hypotensive agents in patients with essential hypertension.

As hypotensive agents, Serpasil alone or in combination with Apresoline was used.

Experimental

Method

Of the patients with essential hypertension, who were admitted in the wards of Sendai National Hospital and were measured for their cerebral circulation and metabolism by N_2O method after stabilization of the blood pressure in about a week after their hospitalization, 11 cases with the blood pressure gently lowered in 9–60 days, in 22 days on an average, by continued oral administration of hypotensive agents, were selected, and the cerebral circulation and metabolism were measured for comparison with

the values before the use of the agents. The subjects thus selected ranged between 43 and 65 years, 54 years in mean age, were free of any complicating cardiac, renal or cerebral vascular disturbances and comprised 4 cases of I grade, 5 cases of II grade and 2 cases of III grade of the retinal findings according to Keith-Wagener's classification, thus consisting in rather mild cases.

These subjects were divided into the group of 7 cases administered with Serpasil alone and the group of 4 cases treated with Serpasil in combination with Apresoline. Method of measurement and units of the values concerning cerebral circulation and metabolism used in the present report are as detailed in the first report[1].

Results

1. *The effect on the cerebral circulation and metabolism of administration of Serpasil alone*

The values concerning the cerebral circulation and metabolism of 7 cases with essential hypertension before and after lowered blood pressure by administration of Serpasil alone are shown in comparison in Table I. The age of these subjects ranged between 43 and 61, averaging 52 years; 3 of them were in I grade group, 2 in II grade group and 2 in III grade group of retinal findings according to Keith-Wagener's classification. Sepasil was administered daily 0.4–0.8 mg., for 9–33 days, for 20 days on an average.

MABP was found lowered in all the cases, by -23.1% on an average, while CBF was found risen in 4 (cases 1, 2, 3 and 5), fallen in 2 (cases 4 and 6) but remained unchanged in 1 (case 7), showing a slight increase of $+10.3\%$ on an average. CVR was found lowered in all the cases, by -28.7% on an average. $(A\text{–}V)O_2$ was augmented in one case (case 6) alone, remaining unchanged or slightly lowered in all the others, so that in mean, no change was found. $CMRO_2$ was elevated in 4 cases (cases 1, 2, 5 and 7), remained unchanged in 2 (cases 3 and 6) and reduced in 1 (case 4), thus showing a slight increase of $+9.2\%$ on an average.

2. *The effect on cerebral circulation and metabolism of administration of Serpasil in combination with Apresoline*

Table II shows the changes of values concerning cerebral circulation and metabolism in 4 cases with essential hypertension after blood pressure reduction due to the combined use of Serpasil and Apresoline. The age range of those patients were between 45 and 65, averaging 58 years; 1 of them was in I grade, 3 in II grade of Keith-Wagener's classification. 0.4–0.8 mg. of Serpasil and 60–200 mg. of Apresoline were applied daily for 19–60 days, 32 days in mean.

MABP was reduced in all the cases, by -18.8% on an average.

TABLE I

The Effect of Administration of Serpasil on Cerebral Circulation and Metabolism

Case	Name	Age	K.W.		MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$	Dosage
1	M.H.	51	I	B	156	48.6	3.21	6.46	3.14	
				A	130	59.8	2.17	6.30	3.77	0.8 mg. for 17 days, 0.6 mg. for another 16 days
2	K.N.	49	I	B	150	40.4	3.71	7.28	2.94	
				A	123	52.0	2.37	6.81	3.54	0.7 mg. for 9 days
3	S.T.	61	I	B	127	51.2	2.48	6.73	3.45	
				A	112	60.0	1.87	6.05	3.63	0.6 mg. for 17 days, 0.8 mg. for another 5 days
4	K.E.	43	II	B	133	75.2	1.77	5.87	4.41	
				A	90	58.7	1.53	5.83	3.42	0.4 mg. for 27 days
5	K.S.	54	II	B	113	56.2	2.00	4.76	2.67	
				A	90	81.6	1.12	4.65	3.79	0.4 mg. for 15 days
6	S.W.	53	III	B	166	40.5	4.14	4.41	1.79	
				A	111	32.4	3.41	4.95	1.60	0.4 mg. for 12 days
7	K.O.	56	III	B	158	53.5	2.95	4.14	2.21	
				A	112	58.6	1.91	4.63	2.71	0.4 mg. for 12 days
	Mean			B	143	52.2	2.89	5.66	2.94	
				A	110	57.6	2.06	5.60	3.21	
	Change				−23.1%	+10.3%	−28.7%	−1.1 %	+9.2%	

B: Values before administration. A: Values after administration.
K.W.: Keith-Wagener's grade of retinal findings.

CBF increased in all the cases, by +57.8% in mean. CVR showed marked fall in all the cases, by −46.6% on an average. $(A-V)O_2$ remained unchanged in 2 cases (cases 9 and 11), but fallen in 2 (cases 8 and 10), thus by −14.3% on an average. $CMRO_2$ was found increased in 2 (cases 8 and 11) but unchanged in 2 (cases 9 and 10), showing an increase of +29.1% on an average.

As shown in Table I and II, a more perceptible improvement was seen in cases administered with Serpasil in combination with Apresoline than in the cases given with Serpasil alone. In Fig. 1 are shown the changes in percentage of individual values for cerebral circulation and metabolism following the blood pressure reduction by those agents, together with the retinal findings according to Keith-Wagener's classification. In all cases with Keith-Wagener I, the administration of Serpasil alone or combined with Apresoline, is followed by high percentages in the increase of CBF and $CMRO_2$ and in the decrease of CVR, while the improvement of those values are lessened, as the retinal findings were deteriorated to the II and III grade. In II and III groups of Keith-Wagener there were found even

TABLE II

The Effect of Administration of Serpasil in Combination with Apresoline on Cerebral Circulation and Metabolism

Case	Name	Age	K.W.		MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$	Dosage
8	S.K.	59.	I	B	155	36.1	4.29	5.37	1.94	
				A	122	73.3	1.66	4.09	3.00	Apresoline 150 mg. for 35 days, Apresoline 150 mg.+Serpasil 0.6 mg. for 25 days
9	K.K.	45	II	B	133	38.2	3.48	5.29	2.02	
				A	107	44.7	2.39	5.41	2.42	Serpasil 0.6 mg. for 13 days, Serpasil 0.6 mg.+Apresoline 60 mg. for 6 days
10	T.A.	65	II	B	124	59.9	2.07	4.60	2.76	
				A	104	95.4	1.10	3.12	2.98	Serpasil 0.6 mg.+Apresoline 100 mg. for 12 days, Serpasil 0.8 mg.+Apresline 200 mg. for 30 days.
11	K.A.	64	II	B	118	40.2	2.94	3.43	1.38	
				A	99	61.6	1.61	3.37	2.08	Serpasil 0.8 mg. for 12 days, Serpasil 0.8 mg.+Apresoline 100 mg. for 7 days
	Mean			B	133	43.6	3.17	4.67	2.03	
				A	108	68.8	1.69	4.00	2.62	
	Change				−18.8%	+57.8%	−46.6%	−14.3%	+29.1%	

B: Values before administration. A: Values after administration.
K.W.: Keith-Wagener's grade of retinal findings.

cases with lowered CBF and $CMRO_2$, when administered with Serpasil alone.

Case Report

Further details on one of the cases that showed improvement and one that showed deterioration, following the lowered blood pressure by administration of the hypotensive agents are demonstrated in the following. The case with a marked improvement was a male of 59 years of age, in Keith-Wagener's I grade and was given daily 150 mg. of Apresoline for 35 days, then 150 mg. of it in combination with 0.6 mg. of Serpasil for 25 days. MABP fell by −21.2% from 155 to 122, CVR was lowered markedly by −61.3% from 4.29 to 1.66, CBF showed significant increase by +103% from 36.1 to 73.3 and $CMRO_2$ also an increase by +54.6% from 1.94 to 3.00—thus an evident improvement being demonstrated.

The case deteriorated after the treatment was a male of 53 years,

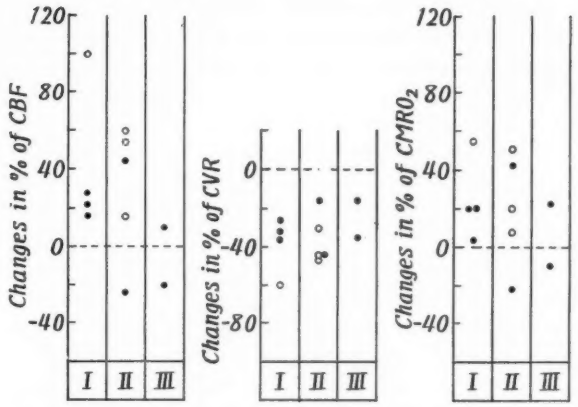

Fig. 1. Relations between retinal findings and changes in individual values for cerebral circulation and metabolism after the administration of hypotensive agents.

● Case administered with Serpasil.

○ Case administered with Serpasil in combination with Apresoline.

I, II, III: Keith-Wagener's grade of retinal findings.

in Keith-Wagener's III grade. He was given daily 0.4 mg. of Serpasil for 12 days. In this case MABP was markedly fallen, by −33.1%, from 166 to 111, but CVR fell only slightly by −17.6%, from 4.14 to 3.41, CBF and $CMRO_2$ both decreased, the former by −20.0%, from 40.5 to 32.4 and the latter by −10.6%, from 1.79 to 1.60. The patient soon fell into cerebral softning showing apparent left hemiparesis. This is one of the remarkable cases which showed an apparent aggravation after the hypotensive treatment.

Discussion

It is a practically important problem whether or not lowering blood pressure using hypotensive agents acts beneficially upon the cerebral circulation and metabolism in patients with essential hypertension.

The result, of course, may be different according to the grade of the disease, the existance of complications, the kind of hypotensives used, their dosis and mode of administration and besides the degree of lowering the blood pressure. In the cases with essential hypertension without any eminent cardiac, renal or cerebrovascular disturbances selected for the present study, the mild lowering of blood pressure induced by prolonged oral administration of Serpasil alone or in combination with Apresoline resulted in relaxation of cerebrovascular spasm with depression of CVR, and excluding a few exceptinal cases, gave rise to increase in CBF and

$CMRO_2$ in the majority of patients and brought about a tendency to return to normal. Considering that the change generally observed in the cerebral hemodynamics in essential hypertension consists in the rise of CVR[2)3)4)], further the lowering of CBF and $CMRO_2$ is often found[5)6)11)] accompanied with advance of the disease, such improvement of the cerebral circulation and metabolism due to the administration of the hypotensives seems likely to exert favourable influence on the function of the brain in the patients with essential hypertension and to be effective in preventing aggravation of the disease and occurence of complications.

Between the results obtained by single administrration of Serpasil and those obtained by combined use of Serpasil and Apresoline, there could be demonstrated some difference. Of course, some difference in the grades of retinal findings was found between both cases investigated, but taking even this into consideration, the fact could be demonstrated that the combined use of those durgs was more effective in improving the cerebral circulation and metabolism, comparing the cases, in which the blood pressure reduction occurred to the same extent. Namely, while in cases administered with Serpasil alone, a lowering of CVR and a slight increase of CBF and $CMRO_2$ were observed, in the cases given with Serpasil in combination with Apresoline a more marked fall of CVR, a more marked increase of CBF and a moderate increase of $CMRO_2$ were found, in particular, the striking augmentation of CBF was worthy of special mention here.

There are only a few reports on the effect of Serpasil on cerebral circulation and metabolism. Goto[7)] studied the acute effect of lowering blood pressure by intravenous injection of Serpasil in 4 cases with essential hypertension and found that CBF was slightly increased in average value but the mean $CMRO_2$ remained unchanged. Upon closer examination, his results show that both CBF and $CMRO_2$ increase in the cases with mild retinal findings, but remain unchanged or decreased in the cases with advanced retinal changes—results in good agreement with the present investigation. Kleh and Fazekas[8)] reported that no significant change was found in the cerebral circulation and metabolism by oral administration of Serpasil, but their subjects were severe cases of hypertensive arteriosclerotic syndrome. No report on the effect of the combined use of Serpasil and Apresoline has been found, but on the use of Apresoline alone, a series of authors[9)10)11)] have demonstrated marked increase in CBF after its administration, either by injection or per os; McCall[10)] alone reporting on an increase of $CMRO_2$ besides CBF.

The results of the present investigation with the combined use of Serpasil and Apresoline showing marked increase of CBF and increase of $CMRO_2$ seem to suggest an additive action fo the two agents. At any rate, the improvement of CBF and CVR induced by these hypotensives

indicates that they are valuable in preventing aggravation and occurence of cerebral complications in essential hypertension, and even the increase of $CMRO_2$ found often indicates that those agents may be effective at least in some cases in improving the anomalies in cerebral metabolism.

Thus, those hypotensives should be noted as of therapeutic significance against reversible hypofunction of the cerebral cells.

Now, upon studying the effect of the hypotensives with consideration to the grade of the retinal findings in individual cases, an improvement of cerebral circulation and metabolism was found in most of the cases with mild retinal changes, but no improvement or even aggravation was noted in some cases with advanced retinal changes. Upon closer examination of the findings of 2 cases aggravated by administration of Serpasil alone, it must be noted that the cases were in II and III grade group of Keith-Wagener's retinal findings, respectively, and showed the high percentages of -32.3% and -33.1% in the drop of MABP, pressure drops much more marked than in any other subject who showed improvement.

From the above results obtained by the present investigation, the following conclusions may be drawn: In the cases with essential hypertension with mild retinal changes, prolonged oral administration of hypotensive agents may be expected to alleviate the changes in the cerebral circulation and metabolism, but in the cases with advanced retinal changes, sometimes no such improvement can be expected for and among the cases showing too conspicuous fall of the blood pressure after administration of the agents, even aggravation of the disturbance in the cerebral circulation may be caused thereby, sometimes leading to cerebral softning or other serious complications. For preventing such a mishap, much caution should be paid to avoid to lower the blood pressure excessively, taking the grade of retinal findings as index.

Summary

For studying the effects of lowered blood pressure induced by prolonged oral administration of hypotensive agents on the cerebral circulation and metabolism in essential hypertension, 7 cases with essential hypertension were administered with Serpasil alone and 4 cases with Serpasil in combination with Apresoline and the values concerning the cerebral circulation and metabolism before and after lowering blood pressure by those drugs were studied in comparison.

1. Following lowered blood pressure, cerebral vascular resistance (CVR) was found fallen in all the cases, cerebral blood flow (CBF) and cerebral oxygen consumption ($CMRO_2$) risen in most of the cases, showing a tendency to return to normal findings, but in a few cases of them CBF and $CMRO_2$ were found unchanged or decreased.

2. The combined use of Serpasil and Apresoline was found more effective in improving the cerebral circulation and metabolism than the single use of Serpasil.

3. In general, the improvement in the cerebral circulation and metabolism following lowered blood pressure was more marked in the cases with mild retinal findings and less in those with advanced retinal findings, and in 2 cases with marked reduction of the blood pressure was observed an aggravation of cerebral circulation and metabolism and one of them was followed by complication with cerebral softning.

4. Details on one of the cases that showed improvement and one that showed deterioration after lowering blood pressure were given.

5. It was infered that for preventing serious complications by use of hypotensive agents much caution should be paid to avoid to lower the blood pressure excessively, taking the grade of retinal findings as index.

The expense of this study was in part defrayed by a Grant in Aid ofr Medical Research, Welfare Ministry. The author wishes to express his thanks to Dr. S. Sato, chief doctor of our Medical Clinic for co-operation in carrying out this investigation.

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Studies on Cerebral Circulation and Metabolism in Hypertensive Diseases

IV. Cerebral Circulation and Metabolism in Hypertensive Postapoplectic Patients

By

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It is clinically of importance to investigate the cerebral circulation and metabolism in hypertensive patients with previous cerebral attack in order to clearify the mechanism of cerebral stroke, thus to go far toward solving the problem on prevention and treatment of the disease. From such point of view, the author measured the cerebral circulation and metabolism in those patients and further dealt with obtained results under consideration of clinical pictures including pathological findings in other organs.

Experimental

Method

Cerebral circulation and metabolism were measured by N_2O method on 27 hypertensive postapopletic patients including 24 cases with hemiplegia, 3 cases with bilateral hemiplegia. The duration between measurement and cerebral attack ranged from 7 days to 4 years, about 1 year on an average. The age of subjects varied between 35 and 73 years, 54 years in mean. All examinees had significant arterial hypertension before or at cerebral attack, but they showed a more or less decrease in blood pressure at the time of measurement. Method of measurement, units concerning cerebral circulation and metabolism are as described in the first report.[1)]

Results

Table I shows the comparison of mean values for cerberal circulation and metabolism in hypertensive postapopletic patients with those of patients with essential hypertension and normotensive subjects in the

TABLE I

Mean Values for Cerebral Circulation and Metablism in Hypertensive Postapoplectic Patients and in Subjects With or Without Hypertension

	Cases	Age range	Mean age	MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$
Hypertensive postapoplectic patients	27	35-73	54	116	42.6	2.78	5.80	2.46
Patients with essential hypertension	51	30-69	54	133	50.6	2.75	5.56	2.76
Normotensive subjects	15	32-76	51	96	55.4	1.75	5.64	3.09

similar age range. In hypertensive postapoplectic patients increase in MABP, singnificant decrease in CBF, apparent increase in CVR, slight increase in $(A-V)O_2$ and significant decrease in $CMRO_2$, all in the mean, were noticed, compared with those of normotensive subjects. In comparison with the values in patients with essential hypertension, the mean values in postapoplectic patients represented slight decrease in MABP, significant decrease in CBF, no change in CVR, slight increase in $(A-V)O_2$ and decrease in $CMRO_2$. In the postapoplectic patients remarkable decrease in CBF and $CMRO_2$ has been demonstrated in this comparison. Those patients were divided into 3 groups, namely cerebral bleeding, cerebral softning, both with hemiplegia, and bilateral hemiplegia due to cerebral softning, according to the clinical pictures, and the mean and individual values were compared among them. (Table II, Fig. 1). No apparent difference could be found between cerebral bleeding group and cerebral softning group. But in group of bilateral hemiplegia, significant decrease in CBF, $(A-V)O_2$ and $CMRO_2$, and increase in CVR were

TABLE II

Mean Values for Cerebral Circulation and Metabolism in Postapoplectic Patients

	Cases	Age range	Mean age	MABP	CBF	CVR	$(A-V)O_2$	$CMRO_2$
Cerebral bleeding group	6	48-60	52	118	42.4	2.81	6.20	2.63
Cerebral softning group	18	35-73	53	115	43.8	2.70	6.03	2.60
Bilateral hemiplegia group	3	49-71	61	113	32.2	3.19	3.62	1.26

evident in comparison with those in other groups. Relations between those values and several clinical findings are as illustrated in Figs. 2–5.

In cerebral softning group, no difference was detectable in CBF

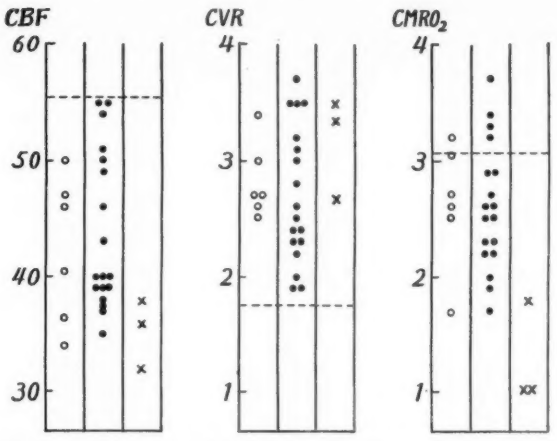

Fig. 1. Individual values for cerebral circulation and metabolism in hypertensive postapoplectic patients.
○ Case with hemiplegia due to cerebral bleeding.
● Case with hemiplegia due to cerebral softning.
× Case with bilateral hemiplegia due to cerebral softning.
---: Mean values in normotensive subjects.

between the groups of O grade (no change) and I grade of retinal findings according to Keith-Wagener's classification, while in II grade group, decrease in CBF was found more often than in the former groups. In cerebral bleeding group, decrease in CBF was found more frequently in II grade group than I grade group, and CVR in both groups showed more marked increase with advance of the retinal findings. $CMRO_2$ showed no correlation with retinal finding in general. (Fig. 2)

In the group with cardiac hypertrophy demonstrated in X ray examination among the cases of cerebral softning, there are found more frequently cases of decrease in CBF and $CMRO_2$ and increase of CVR. Comparison was unable among cases of cerebral bleeding, as all of them showed cardiac hypertrophy in X ray examination without exception. (Fig. 3)

In both of cerebral softning and cerebral bleeding group, apparent decrease in CBF and $CMRO_2$ were seen more repeatedly in group with ECG findings, such as myocardial damage, coronary insufficiency, left ventricular hypertrophy, than in group without ECG changes. (Fig. 4)

No correlation could be found between age and CBF, CVR and $CMRO_2$ in both of cerebral bleeding and softning group.

With respect to the disturbance of motor function, cerebral circulation and metabolism were compared in two groups: cases with and without

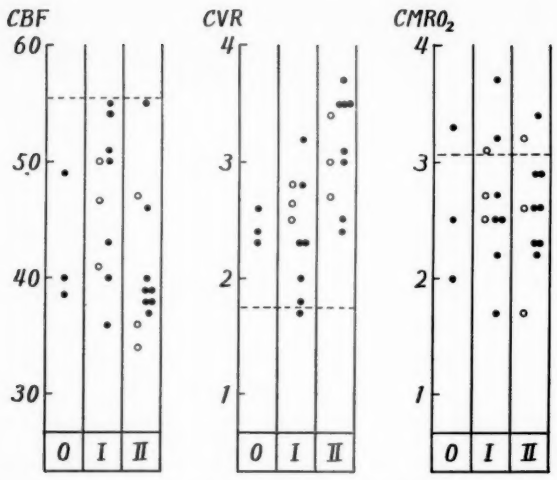

Fig. 2. Relations between retinal findings and cerebral circulation and metablism.
○ Case with hemiplegia due to cerebral bleeding.
● Case with hemiplegia due to cerebral softning.
0 I II: Keith-Wagener's grade of retinal findings.
---: Mean values in normotensive subjects.

the disturbance of walk. (Fig. 5) In cerebral softning, any difference could not be detected between two groups. In cerebral bleeding, the patients showed lowered CBF and $CMRO_2$ in group with disturbance of walk, but the number of cases was too small for any definite conclusion.

Discussion

1. *General consideration on the cerebral circulation and metabolism in the hypertensive postapoplectic patients*

Reports have been made on the cerebral circulation and metabolism in this disease by a series of authors (Scheinberg[2], Heyman[3], Atarashi[4], Mashima[5], Kato[6], etc.) The results stated in the present paper are nearly in accordance with them in general, yielding the occurrence of severe disturbances in cerebral circulation and metabolism. CVR, nevertheless, did not surpass that of hypertensive patients without cerebral attack in our cases, presumably because their MABP was comparatively low in general at the time of measurement. In the present investigation no apparent difference in cerebral circulation and metabolism could be found between cerebral hemorrhage and cerebral softning, in agreement with the results by Aizawa[7] and Kato[6].

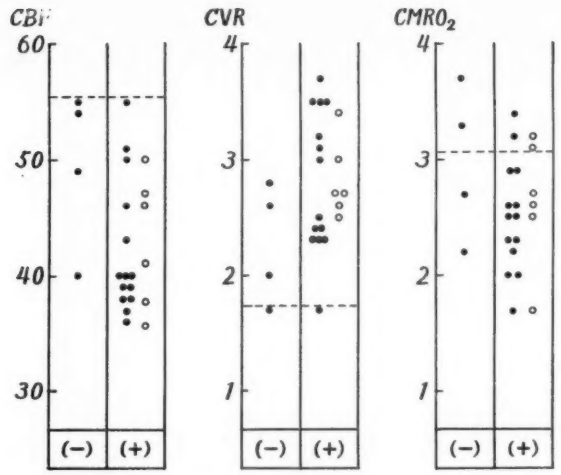

Fig. 3. Relations between X-ray findings in heart and cerebral circulation and metabolism.

○ Case with hemiplegia due to cerebral bleeding.

● Case with hemiplegia due to cerebral softning.

Group (−): Cases without left ventricular hypertrophy.

Group (+): Cases with left ventricular hypertrophy.

---: Mean values in normotensive subjects.

It is interesting but reasonable evidence, that cerebral circulation and metabolism in patients with bilateral hemiplegia caused by repeated apoplectic insults represented more severe changes than in patients with only one insult. This result corresponds with that by Heyman[3]. In patients with hemiplegia, $(A–V)O_2$ was increased in general, going hand in hand with the decrease of CBF, thus endeavoring to maintain $CMRO_2$ near to normal level, though the values of $CMRO_2$ ranged lower than that in hypertensive non-apoplectic patients, indicating the reduced function of the brain tissues. An interesting exception is that $(A–V)O_2$ in patients with bilateral hemiplegia did not increase any more in spite of marked decrease in CBF. It seems that in these cases the cellular damage in brain tissue inhibits the proper oxygen consumption or the oxygen demand diminishes as the result of its lowered metabolic rate.

As N_2O method yields average values of blood flow and metabolism in all parts of the brain, a localized damage does not greatly influence on the data measured by this method, so far as the damage is severe and the blood flow in that part is negligible. Accordingly, the abnormalities observed in those patients support the existence of brain insury of some

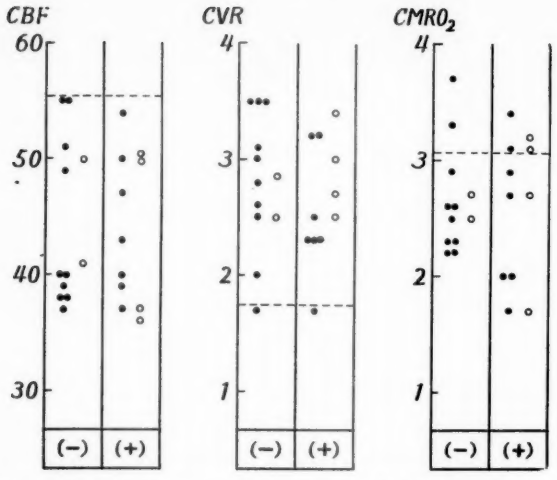

Fig. 4. Relations between electrocardiographic findings and cerebral circulation and metabolism.
○ Case with hemiplegia due to cerebral bleeding.
● Case with hemiplegia due to cerebral softning.
Group (−): Cases without ECG findings such as myocardial damage, coronary insufficiency or left ventricular hypertrophy.
Group (+): Cases with those ECG findings.
---: Mean values in normotensive subjects.

extent as a whole caused by profound vascular changes, or diffuse compression or edema in the surrounding area around the hemorrhagic focus in the brain.

As mentioned in the first report[1], hypertensive patients complicated with cerebral arteriosclerosis show disturbances to some extent in cerebral circulation and metabolism, which seem to approach the abnomral values in post-apoplectic patients. Among those factors which concern to the development of disturbances in cerebral circulation and metabolism in postapoplectic patients, cerebral arteriosclerosis cannot be attributed to have occured after apoplexy, but is logically assumed to exist preceedingly and gives rise to apoplexy, when it develops to a certain degree and some motivating factors are added to it. In the third report[8] the author dealt with a hypertensive case with cerebral arteriosclerosis which showed disturbances in the cerebral blood flow and metabolism, exaggereted after lowering blood pressure by administration of hypotensive agent, and was complicated with cerebral softning soon thereafter. This case seems to support the above assumption on the mechanism of cerebral softning. It is obvious that there is a close relationship between cerebral softning and

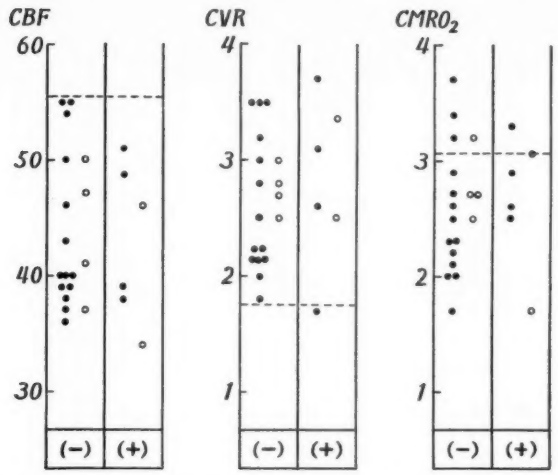

Fig. 5. Relations between degree of disturbance of motor function and cerebral circulation and metabolism.
○ Case with hemiplegia due to cerebral bleeding.
● Case with hemiplegia due to cerebral softning.
Group (−): Cases without disturbance of walk.
Group (+): Cases with disturbance of walk.
- - -: Mean values in normotensive subjects.

cerebral arteriosclerosis. On the other hand, cerebral bleeding affects the brain tissues of surrounding area, causing edema and compression, and its effect on the cerebral hemodynamics may be much more complicated than in case of cerebral softning. Considering the results of anatomical studies, which clarified that, in cerebral hemorrhage so much close relationship between cerebral attack and cerebral arteriosclerosis could not be detected as in cerebral softning, the mechanism of cerebral hemodynamics in post-hemorrhagic brain damage can not be attributable to the same as in case of simple lack of blood supply in the brain. Other factors including that as mentioned above must contribute to the development of circulatory and metabolic disturbances in cerebral hemorrhage. Consequently, the mechanism of outbreak of cerebral bleeding cannot be estimated by this method and it remains as a question. Investigation must be carried out to reply this question by measuring cerebral blood flow and metabolism not only on postapoplectic patients but also on individuals before and after cerebral bleeding in comparison.

2. *Clinical findings and cerebral circulation and metabolism in postapoplectic patients*

The obtained data in cerebral circulation and metabolism as men-

tioned above were related to retinal findings and cardiac findings to some degree in postapoplectic patients. But the correlation was not so close as in hypertensive patients without apoplexia, as stated in the second report[9].

No relations could be found to age. These findings seem to indicate that cerebral circulation and metabolism in those patients are peculiarly influenced or decided by intense vascular damage in the brain or changes in brain tissue, rather than by other factors, compared with the case without the complication of apoplexy.

No significant difference could be demonstrated between 2 groups, with and without severe motor disturbance. This seems reasonable because those motor disturbances are usually caused by localized severe brain damage, which might not affect the cerebral circulation and metabolism measured by N_2O method any more as only a scarce blood stream would be remained there.

Summary

On 27 hypertensive postapoplectic patients, 24 of which were complicated with hemiplegia and 3 of which were complicated with bilateral hemiplegia, cerebral circulation and metabolism were measured by N_2O method.

1. The disturbances in cerebral circulation and metabolism were exaggerated in those patients compared with hypertensive patients without apoplectic attack or normotensive persons.

2. No significant difference in cerebral circulation and metabolism could be detected between cerebral softning group and cerebral bleeding group. In patients with bilateral hemiplegia, which had cerebral attacks repeatedly, the most apparent disturbances could be found in cerebral circulation and metabolism.

3. A correlation to some extent could be detected between cerebral circulation and metabolism on the one hand and retinal findings or cardiac findings on the other, but it was not remarkable compared with that in hypertensive, non-apoplectic patients. No parallelism could be found between age and the cerebral circulation and metablism. Differences between 2 groups, with and without severe motor disturbances were not significant.

4. The cause of disturbances in cerebral circulation and metabolism in postapoplectic patients was discussed, the correlation between those changes in them and clinical findings being closely examined.

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The Spatial Distribution of Electric Responses to Focal Illumination of the Carp's Retina

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Introduction

The structural organization for lateral interaction of the vertebrate retina is well-known. The classical experiments by Adrian and Matthews[1], Granit[2] and others may be taken as evidence for lateral interaction, if responses recorded really came from the area intentionally illuminated. Fry and Bartley[3] maintained that electroretinogram was mainly a response to stray light within the retina, and this theory was supported by Boynton and Riggs[4], but opposed by Granit, Rubinstein and Therman[5]. Evidence was, however, provided that an electric response to a small, localized illumination of the retina is a focal effect (Tomita, Mizuno and Ida[6], Tomita and Torihama[7] and Brindley[8]).

Marg and Heath[9] noted some evidence of interaction with two stimulus spots spaced 0.1–1 mm. apart on the frog's isolated retina. Brindley[10] frequently observed that the response of the inverted preparation of the frog's retina was negative-going within the illuminated area, but positive-going outside it, and inferred that the structures generating the responses were tangentially oriented in the retina, providing sinks for current within the illuminated area and sources outside it.

We made similar observation in the isolated preparation of the carp's retina. In the present investigation experimental conditions favorable and unfavorable for this phenomenon were studied systematically, and made it probable that the potential of opposite polarity outside the illuminated area was due to physiological interaction.

Experimental

Methods

The excised eye of the carp (Cyprinus carpio) was used exclusively. After

removal of the sclera, the cornea and lens were cut away together in one piece. The remaining part was mounted with the receptor side upwards on a piece of Ringer-soaked black cloth 2×2 cm. in size covering a silver plate which served as a diffuse electrode. Then the chorioid and pigment epithelium were scraped away as gently as possible not to give any light pressure to the underlying structure. Since the receptor cells in the inverted preparation were in direct contact with the air, steady responses could be obtained for about 2 hours at room temperature without any special oxygen supply. Rapid deterioration was, however, noticed at temperatures higher than 22°C, so that all the experiments were carried out at temperatures between 12 and 20°C.

As a recording electrode a 3 *M* KCl-filled micropipette about 1 μ in tip diameter was used, and its position in the retina was measured by a micrometer-gauge fitted to a micromanipulator. The recording equippment used consisted of a D. C. amplifier with a cathode-follower input and a dual beam oscilloscope. The optical system used is illustrated schematically in Fig. 1. To make stimuli

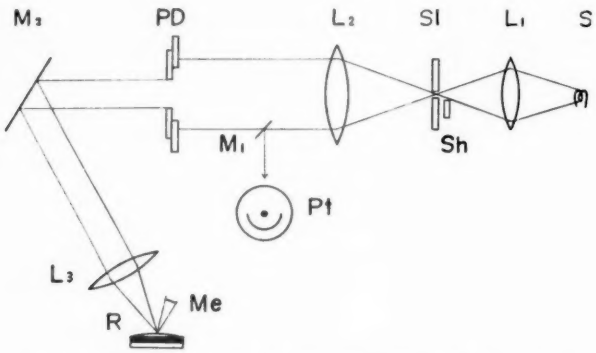

Fig. 1. Optical arrangement for microillumination of the inverted retina. L_1, L_2 and L_3 lenses; M_1 and M_2 plane mirrors; Me a microelectrode; PD a patch with a figure cut in it together with a diaphragm; Pt photocell for signalling a stimulus; R retina; S light source; Sh shutter; Sl slit.

of various sizes and shapes, a black patch P with a corresponding figure cut in it was placed at the diaphragm D, which was moved by a micrometer parpendicularly to the light-path, when it was necessary to move the image of the figure focused on the retina.

Results

The carp's retina usually exhibits a response of a complicated pattern to even illumination, when the potential is recorded with large electrodes placed on both sides of the retina. The pattern of potential is much simpler for focal illumination when recorded with a microelectrode. Examples are shown in the inset of Fig. 2. In this experiment a spot of light 60 μ

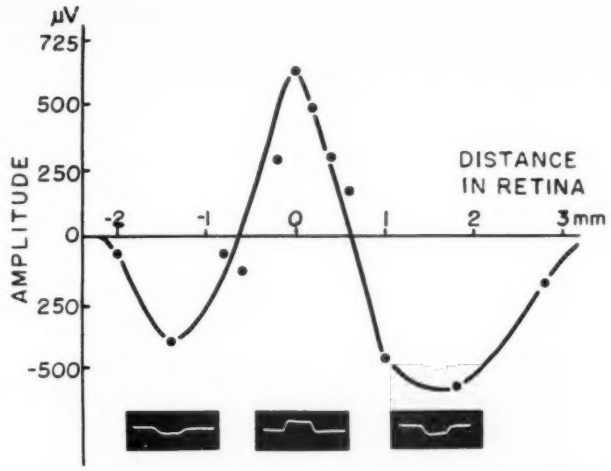

Fig. 2. Lateral distribution of potentials in response to microillumination of 60 μ in diameter centered on the point labeled 0. Positive-going potentials are plotted upwards. Intensity of illumination 7,000 lux. Representative records taken at three different points are shown in inset....

in diameter was used. In these and the other records the upward direction indicates increasing positivity of the microelectrode against the diffuse one in contact with the inner surface of the retina. It is to be noted that at the illuminated part and its immediate neighborhood the potential is positive, while in the surrounding areas it is negative. The distribution curve was mapped in such a way that the test spot of light was moved step by step along a straight line across the fixed electrode to take a record for each position of the test spot. The magnitudes of responses as measured from the baseline to the steady level of potential were plotted as ordinates against distances from the electrode tip to the center of the illuminated part. As can be seen in Fig. 2, the positive potential falls off from the center towards the peripheries of the illuminated area and changes its polarity at a certain distance—about 0.6 mm. in this case—from the margin of the light spot. The negative potential reaches a maximum at a distance of about 1.5 mm. and extends as far as about 3 mm.

In another series of experiments the light spot was fixed at a certain point, and the microelectrode was moved step by step along a straight line passing through the illuminated part. The results obtained were found to be essentially the same as that illustrated in Fig. 2.

The pattern of potential was a little more complicated, when the size of illumination was greater. In the experiment shown in Fig. 3, a

rectangular stimulus 0.4×1 mm. in size was used, and responses were measured along the straight line parpendicular to the long axis of the rectangular image. In A of Fig. 3 are illustrated records taken at various distances from the illuminated part. The record taken at the center of the illuminated area (record 0.00) consists of a phasic component and a tonic or steady one. At cessation of illumination an " off " response composed of a negative potential is seen. Negative potentials recorded at far distances from the illuminated part consist of a negative steady component alone. In B of Fig. 3 potentials obtained at the center of the illumi-

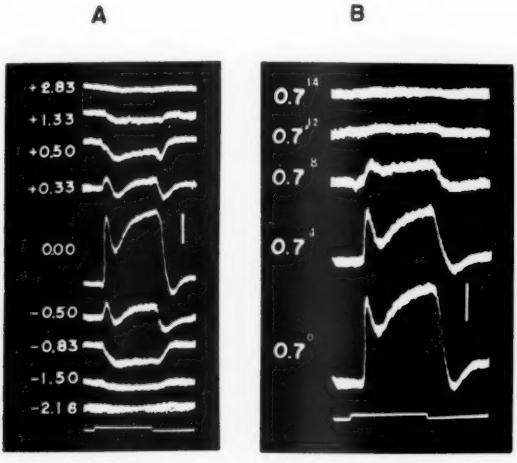

Fig. 3. A: Potentials recorded at various distances from the locus exposed to a stimulus 0.4×1 mm. in size and of a fixed intensity 7,000 lux. B: Potentials recorded from the area exposed to illumination of varying intensities with 7,000 lux as unit. Upward direction is positive. A stimulus 0.9 sec. in duration is indicated by upward shift of horizontal line below records. Vertical bars indicate 1 mV.

nated area in response to stimuli of varying intensities are shown. It is to be noted that at the site of illumination no negative potential could be obtained with mere reduction of the intensity of illumination. This fact is important for interpretation of the negative potential in the surrounding areas, for one may suppose that the negative potential in question can be caused by stray light, but such an interpretation is untenable inasmuch as no negative potential is produced at the site of illumination even if its intensity is made comparable to that of stray light. The phasic component which is very conspicuous in the records taken at the illuminated part is almost missing in the records taken at farther distances. In view

of this finding it seems almost impossible to account for the negative potential in the surrounding area in terms of electrotonic spread from the illuminated area.

Area-effects and effects of light adaptation

In A of Fig. 4 the effect of changing the area of illumination is shown; when the diameter of the light spot was less than 1 mm. the potential was

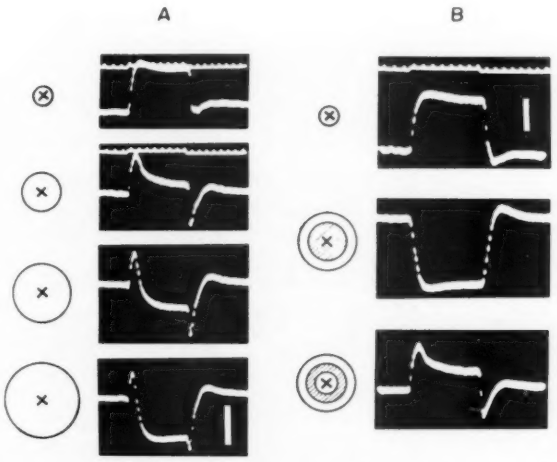

Fig. 4. Area-effects. In A light spots used were 1, 2, 3 and 4 mm. in diameter. Position of microelectrode is marked by cross. In B middle record indicates effect of an annular stimulus. Bottom record indicates result obtained by simultaneous exposure to spot and annulus. Intensity of illumination was 2,160 lux. Vertical bar indicates 1 mV.

always positive-going, almost rectangular in shape, but phasic components at " on " and at " off " were predominant with larger spots of light. The steady component was positive for smaller light spots, but negative for large ones. In B of Fig. 4 the effects of a light spot and an annular stimulus are compared. The potential at the center of the annulus was always negative in contrast to the positive potential at the center of the light spot. It should be remembered that the magnitude of the negative potential is even greater than that of the positive one. The bottom record represents the result of simultaneous exposure to the spot and the annulus. The following characteristics can be seen from this record: 1. Remarkable phasic components are seen, although they were very slight in separate exposure. 2. The resultant steady component is positive, although the positive component was smaller than the negative one in isolated stimula-

tion. These findings show that the resultant is not a mere algebraic sum of both potentials.

The negative potential in the surrounding area is not always manifest, especially under dark adaptation. When the photosensitivity of the retina is high, it will respond to such a weak stimulus as stray light and give rise to a positive-going potential which in turn masks the negative-going potential induced there. A series of records in Fig. 5 A will serve to show

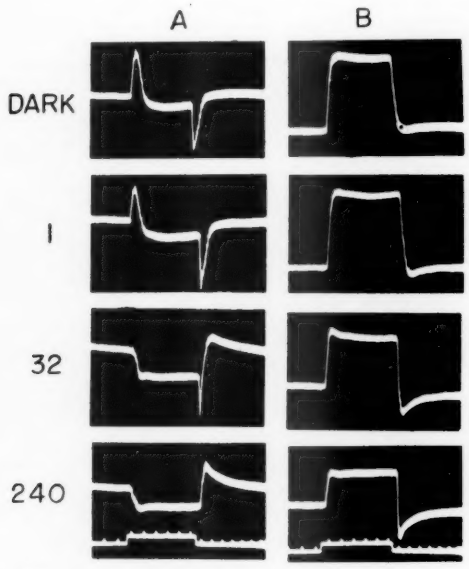

Fig. 5. Effects of light adaptation upon positive and negative potentials. Series A was obtained at the center of an annulus with inner and outer diameters 3 and 4 mm. respectively. Series B was recorded at the center of an illuminated area 0.4×0.4 mm. Intensity of background illumination is given in lux on left side of each record. Intensity of light stimulus was 2,160 lux. Time mark 0.1 sec.

the mechanism of the masking effect of stray light. The microelectrode was placed at the center of an annulus whose outer and inner diameters were 4 and 3 mm. respectively. The record taken under dark adaptation represented a pattern of electric interference mainly consisting of phasic components. A slight steady component of negative polarity appeared in the record taken under background illumination of 1 lux. As the level of light adaptation was raised, the steady negative component became more pronounced, and the phasic component less conspicuous. A very simple pattern was observed at adaptation levels higher than 240 lux. This

series of potential variations at the center of the annulus will be explained easily, if it is assumed that the potential observed at lower background illumination represents an interference product of positive and negative steady potentials, and that the positive component due to stray light is diminished with increasing intensities of adapting light. The potentials recorded at an illuminated part of the same preparation are illustrated in Fig. 5 B. They are of similar pattern at all the adpatation levels.

The effects of ageing and temperature

In a fresh preparation we obtained sometimes positive-going potentials alone not only in the illuminated part but also in the surrounding areas. With the lapse of time the same preparation became to exhibit a potential distribution positive in the illuminated area but negative in the surrounding area. An example is shown in Fig. 6. This effect of ageing seems to be due to the different liability of both potentials to drying and other factors. As will be shown in the following section, there is evidence that the positive

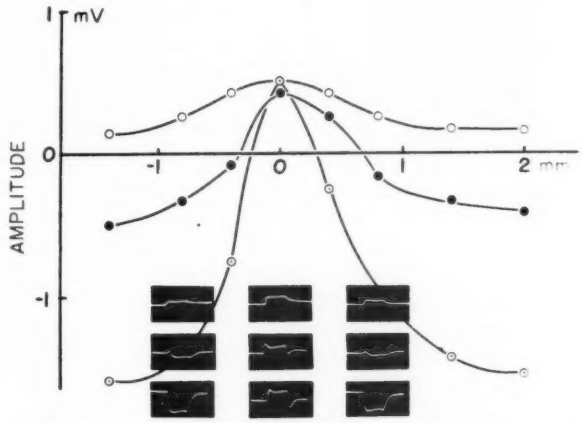

Fig. 6. Effects of ageing upon lateral distribution of potentials. A stimulus 0.4 × 1 mm. in size and of intensity 3,150 lux was centered on point labeled 0. Curves from top to bottom were obtained immediately after mounting of preparation, 15 and 30 minutes later respectively. It took about 2 minutes for mapping each curve.

potential originates in the superficial layer on the receptor side, while the origin of the negative potential lies in the deeper layers. Therefore, the positive potential, especially that caused by weak stray light, will be reduced with time more rapidly than the negative potential in the deeper layers which are protected from external stresses. In consequence, the

resultant potential in the surrounding area becomes negative.

We placed a droplet of saline on a part of the outer surface of the retina to protect this part from drying and found that the droplet itself suppressed the positive potential in response to weak illumination. A droplet of paraffin oil was found to show a similar depressant effect. Far more dramatic was the effect of one per cent solution of cocaine; it abolished a positive potential of an illuminated area in a several seconds. In contrast with this, a negative potential recorded from the dark area within an annular stimulus was enhanced instantaneously. Such an increase of negative potential must have been caused by suppression of the positive component due to stray light.

Both kinds of potential decreased in amplitude at temperatures lower than 10°C. In Fig. 7 distributions of potentials are mapped at two diffe-

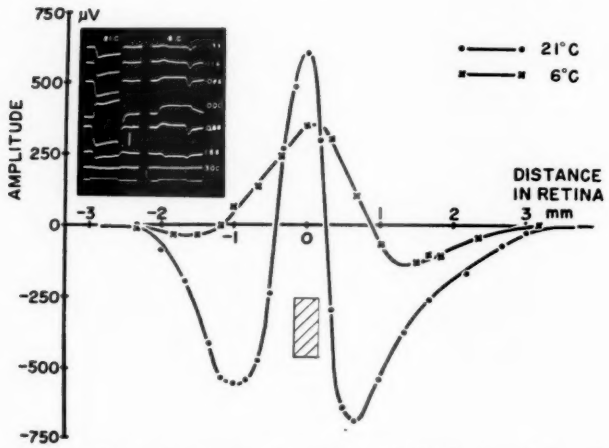

Fig. 7. Effect of temperature upon lateral distribution of potentials. Size and location of illumination are indicated by a shaded rectangle.

rent temperatures 6° and 21°C. As can be seen in this figure, positive and negative potentials are differently affected by lowering the temperature. If both kinds of potential were based on the source-sink relation, temperature dependence would be identical for both potentials.

The effect of a mechanical cut into the retina upon the distribution of potentials

On one side of an illuminated part of the retina a cut was made with a blade of razor, and then the distribution of potentials was determined as usual. As indicated by a continuous curve in Fig. 8 A, positive potentials

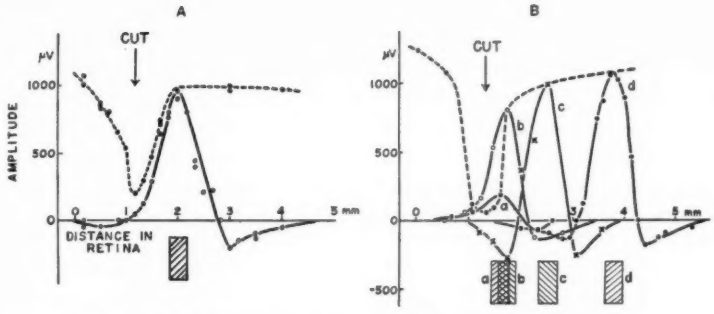

Fig. 8. Effect of mechanical cut upon distribution of potentials. Continuous curves represent distribution of potentials and broken ones indicate responsiveness of each locus in region of cut and its neighborhood. Shaded rectangles indicate positions of illuminated loci relative to cut. Intensity of illumination was 7,000 lux.

are distributed almost symmetrically, but concerning the distribution of the negative potentials remarkable asymmetry is seen. In order to get information about topographical relation of the cut to the illuminated area, a microelectrode was moved horizontally step by step, and a positive response to a spot of light centered on the tip of the microelectrode was recorded at each position. The broken curve in the same figure represents the distribution of responses to such search stimuli. If the magnitude of response serves as a certain measure for local responsiveness, it may be said that the cut was made at the locus of the smallest response as indicated by an arrow. We also see that the damage due to cut fell off with increasing distances on both sides of the cut.

Let us now turn our attention to the continuous curve. As has been mentioned above, the distribution of positive potentials is almost symmetrical, the center of symmetry being the illuminated part which is indicated by a shaded rectangle in the figure. Between the cut and the illuminated area there is no sign of deterioration, as far as the distribution of positive potentials is concerned. This finding seems to contradict the obvious sign of damage as indicated by the broken curve in this region. This apparent contradiction may be explained if it is assumed that the positive potential in this region is due to electrotonic spread from the illuminated part. It deserves attention that the negative potentials almost disappeared on the left side of the illuminated part, while they developed well on the opposite side.

In another series of experiments focal illumination was made at various distances from an injured portion of the retina to map the distribution of potentials for each position of the focal illumination relative to the cut.

The results obtained are illustrated in Fig. 8 B. The locus of the cut was searched as has been described above, and is indicated by an arrow in the figure. The positions of focal illumination are indicated by shaded rectangles labeled *a*, *b*, *c* and *d* respectively. The distribution curves *a*, *b*, *c* and *d* were obtained for the corresponding positions of illumination. As can be seen in this curve, symmetry concerning the distribution of positive and negative potentials is preserved well in curves *c* and *d* which were obtained by focal illumination made at relatively great distances from the injured portion. The symmetry is, however, entirely lost in curves *a* and *b* which were obtained at relatively short distances from the cut; in these curves no negative potential appeared on the left side of the illuminated part in contrast to the well-developed negative potential on the opposite side. For curve *a* the illumination was made at a part so near the injured portion that the positive potentials were reduced below normal. Corresponding to these reduced positive potentials the negative ones induced into the intact area were smaller than normal. On the left side of the illuminated area no negative potential appeared, and instead, the positive one extended farther beyond the cut probably by electrotonic spread.

It is to be noted that the negative potential of curve *c* was of normal magnitude on the left side of the illuminated part and extended over the injured portion. This must be electrotonic spread, too. Thus the electrotonic spread occurs whether or not the retinal area is intact. On the contrary, the negative potential cannot be induced into a damaged part.

Retinal layers responsible for production of positive and negative potentials

The most important fact revealed by the present experiments is that responses of reverse polarity are obtained from an illuminated area and its surrounding area. This is not restricted to inverted preparations of isolated retina. The reversal of polarity can be demonstrated with eyebulb preparations as well.

The microelectrode placed at the center of an illuminated area was moved vertically in steps 50 μ and at each depth a response was recorded. The result obtained is represented by a continuous curve marked by dots in Fig. 9. The positive potential was highest at the surface and in its vicinity and decreased very steeply around an apparent depth of 100 μ. This change of potential depending upon depths was fairly reversible, as indicated by the curve determined in the reverse order. According to histological examinations made by Oikawa, Ogawa and Motokawa[11], receptor cells are included in a range of apparent depths from 0 to 100 μ. Therefore, the receptor layer may be responsible for the production of positive potentials at the illuminated part, although contribution by other

kinds of tissue is not excluded.

In one and the same preparation negative potentials were recorded at a certain horizontal distance from the margin of the illuminated part but at various depths. The result obtained is represented by the broken curve in Fig. 9. In this experiment reversibility was not so satisfactory

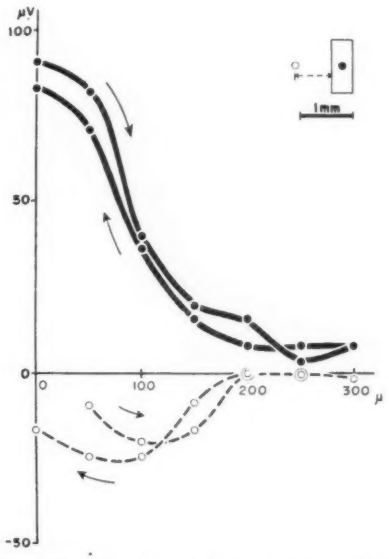

Fig. 9. Dependence of positive and negative potentials on depths from receptor side. Continuous and broken curves refer to positive and negative potentials respectively. A rectangle in inset indicates illuminated area, and positions of electrode for recording positive and negative potentials are marked by dot and open circle respectively.

as in the experiment concerning positive potentials, but it is almost certain that the magnitude of response was maximal around a depth of 100 μ from the receptor side. Similar experiments were carried out with eleven other preparations, and it has been confirmed that the positive and negative potentials originate in different layers of the retina.

In some cases the distribution curve was not so smooth as those illustrated above, some deformation being seen at a certain depth. An example is shown in Fig. 10. The positive potential was replaced by a large negative one at a certain depth, but the latter gave place to a small positive potential on further advancement of the microelectrode. The magnitude of such a large negative potential ranged from a few hundred μV to about 10 mV. It was found that the level of resting potential shifted in the nega-

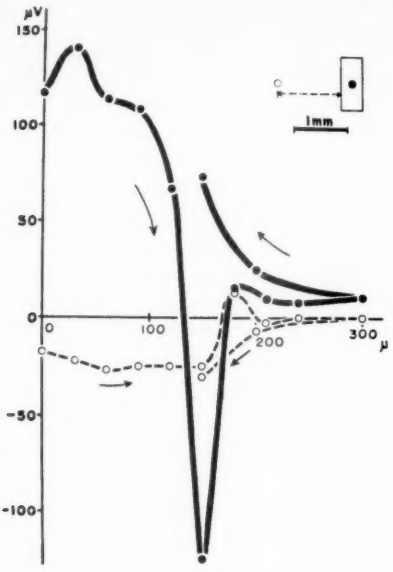

Fig. 10. Dependence of potentials on depths from receptor side. A full account of deformation of distribution curve is given in text.

tive direction just before such a large negative potential appeared. The change of resting potential ranged from a few mV to over 10 mV. In view of its large magnitude and the resting potential shift it must be related with the intracellular slow potential as studied by Svaetichin[12] and by Motokawa, Oikawa and Tasaki[13].

Discussion

Possible factors responsible for potentials recorded outside the illuminated area are stray light, electrotonic spread and physiological spread. Any one of these three factors predominates over the others depending upon experimental conditions, physiological states of the preparation, animal species, etc. The effect of stray light can greatly be reduced by background illumination of sufficient intensity. In our experiments the negative potential in the surrounding area generally increased in amplitude, when the effect of stray light was reduced by suitable background illumination (Fig. 5). It is to be noted that the positive potential recorded in the illuminated area of one and the same preparation was smaller under the background illumination than otherwise. From this finding it is almost apparent that the negative potential in question is not due to stray light.

The effect of stray light prevails in an extremely photosensitive state of the preparation, for example, under dark adaptation. Under such conditions no change in polarity can be found when the electrode is moved from the illuminated locus into the surrounding area. Therefore, the change in polarity is not a constantly observable phenomenon. In fact, Brindley observed it in a limited number of preparations of frog's isolated retina. He tried to explain the observed reversal of polarity by assuming sinks and sources on some tangentially oriented structures such as horizontal cells. From this assumption, it seems, however, difficult to interpret our findings that by lowering the temperature the negative potential was affected more severely than the positive one (Fig. 7). Moreover, the negative potential is not a mirror image of the positive one.

According to Pilz, Sickel and Birke[14], electrotonic spread played no important role under their experimental conditions. In our experiments the extent of electrotonic spread seemed much smaller than that of stray light in accordance with the previous authors. Electrotonic spread in pure form can be seen in Fig. 8 B, where the effect of stray light and physiological spread was eliminated by mechanical injury in some region of the retina. In curves *a* and *b* of the figure it can be seen how the positive potential produced in a less damaged locus illuminated directly spreads electrotonically over a severely damaged part. The extent of spread is about 1 mm. in these cases. Stray light must have made no contribution to the spread of positive potential, because it could not cause any positive potential in the damaged region. It should be emphasized that no negative potential was induced into the injured part and across it. This finding strongly suggests that the negative potential is induced by some physiological mechanism.

We must be careful in generalizing our results to retinae of other species, for we know that the type, size and polarity of electrical responses obtained under similar experimental conditions can differ from species to species, but hope that similar lateral spread of physiological nature will be revealed by suitable analysis in retinae of other species, too.

Summary

Potentials in response to microillumination were recorded with a 3 *M* KCl-filled microelectrode of about 1 μ in diameter in the illuminated and surrounding areas of the inverted preparation of the carp's isolated retina.

1. The shape of an electric response to a small spot of light less than 1 mm. in diameter was almost rectangular, and its polarity was positive at the illuminated part, but negative in the surrounding area. The negative potential was detectable as far as 2 mm. from the margin of the illuminated part.

2. Light adaptation of suitable intensity was favorable for the manifestation of negative potentials in the surrounding area, and this effect is to be ascribed to suppression of the effect of stray light by light adaptation.

3. The negative potential was suppressed at low temperatures below 8°C more strongly than the positive potential.

4. No negative potential was induced beyond a mechanical cut made closely to the margin of the illuminated area.

5. The positive potential at the illuminated locus was highest at the outer surface of the retina, while the negative one in the surrounding area was maximal in amplitude at an apparent depth of about 100 μ from the receptor side.

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Studies on Parenteral Fluid Therapy in Neurosurgery

(First Report)

By

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Though the recent improvement of operative results have contributed much to the development of various parenteral fluid therapies, the parenteral fluid therapy for brain surgery seems not so satisfactorily advanced, for the researches have been limited to the amount or speed of injection. It is an urgent problem that the parenteral fluid therapy for the patients with high intracranial pressure is sometimes dangerous. Patients without high intracranial pressure, though is not so much exposed to the danger as are the cases with heightened cranial pressure, for example, high intracranial pressures with severe headache, nausea and vomiting may often take fatal consequence by inappropriate application of parenteral fluid therapy, because, it applied to such patients, it may produce more irreversible high intracranial pressure.

It is the purpose of this paper to elucidate the relationship of the various kinds of parenteral fluid and cerebral edema or production of cerebraospinal fluid, experimentally and finally to find an ideal fluid which does not incur hyperintracranial pressure.

In the first step of this investigation, cerebral water contents were measured following cerebral tissue emersion into various kinds of fluid experimentally and then, change of the water contents of the brain was observed following injection of intravenous fluids.

Transposition of the various parenteral fluids into the cerebral tissue or the cerebral fluid was investigated by heavy water as a good tracer of the water injected.

Finally the relationship between intracranial pressure and the various parenteral fluid was studied in this experiments.

A. Change of cerebral water contents due to various kinds of parenteral fluid

Experimental Methods and Materials

One hundred and twenty mice weighing about 20 gramms were used and guillotined without anaesthesia.

A section of brain weighing hundred milligramms was taken and put into full moistured chamber and then the section were immersed in various kinds of fluids with serum, for the perid of twenty minutes while preserved in fully saturated oxygen at the temperature of 38°C. The weight of the brain section was measured before and after the immersion and the increment in weight may be taken to indicate the grade of swelling of the cerebral tissue during the immersion.

The kinds of fluids were as follows: (1) 0.9% saline (2) 5% solution of glucose (3) 0.5% glucose in 0.81% saline (abv. 0.5 Gl. 0.81 NaCl) (4) 2.5% glucose in 0.45% saline (2.5 Gl. 0.45 NaCl) and (5) 4.5% glucose in 0.09% saline (4.5 Gl. 0.09 NaCl) (1)–(5) are isotonic solutions.

(6) serum (7) K-solution (0.175% NaCl, 0.065% KCl, 7.5% glucose).

(8) 7.5% glucose 0.45% NaCl, (abv. 7.5 Gl. 0.45 NaCl).

(9) 9.0% glucose in 0.18 saline (abv. 9.0 Gl. 0.18 NaCl).

(10) 1.0% glucose 1.62% saline (abv. 1.0 Gl. 1.62 NaCl).

(11) Periston N " Bayer " (7)–(11) are slightly hypertonic. (12) 20% dextran (13) 20% glucose (14) 50% glucose (12)–(14) are hypertonic.

Similar the same experiments were performed mice 48 hours after experimental unilateral frontal lobe puncture.

Using 160 mice, the various solutions were administered intravenously in the dosis of 40 cc/kg in the rate of 2 cc per minutes.

Sixty minutes after the injection, accurate measurements of one hemisphare each of the brain were performed and then the brain tissue specimens were preserved in thermostatic chamber for the period of forty-eight hours at the temperature of 110°C.

The difference of brain weight before and after preservation showed the change of the cerebral water content. In addition, mice with injured brain were experimented upon in the same method as in the previous investigation in vitro.

Results

In vitro: The initial tissue weight increased by 11% at the highest after immersion in 5% glucose solution by 6–7% in 0.9% saline, 4.5 Gl. 0.09 NaCl, 7.5 Gl. 0.45 NaCl and 2% by 2.5 Gl. 0.45 NaCl, K-solution, Periston-N, and serum.

Increase by 1% was found by immersion in 1.0 Gl. 1.62 NaCl. and 20% dextran.

Following immersion in 20% glucose or 50% glucose solution, the

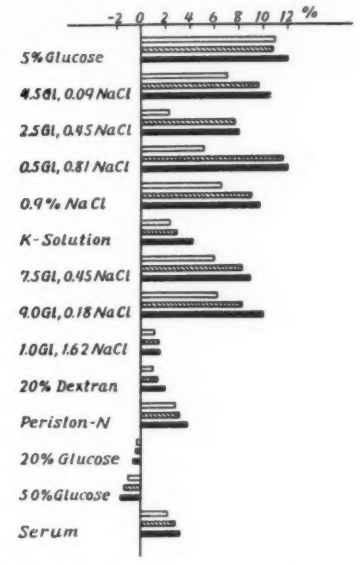

Fig. 1. Swelling of brain tissue in various parenteral fluids. White column : intact mice, shaded column : non-injured side of injured brain mice, black column : injured side of injured brain mice.

cerebral water content was found decreased.

In the case of brain injured mice, the grade of the cerebral swelling was generally higher than that of non-injured mice, and also the brain tissue of the injured side has showed stronger swelling than that of the other side.

Cerebral swelling was observed to the degree of 11 to 12% of the initial weight in maximum in 5% Gl. or 0.5 Gl. 0.81 NaCl, and 9 to 10% by 4.5 Gl. 0.09 NaCl, 0.9 NaCl, 7.5 Gl. 0.45 NaCl and 9.0 Gl. 0.18 NaCl solutions.

K-solution, Periston-N as well as serum caused the cerebral tissue swell by 3%.

Dehydration was observed by use of 20% glucose or 50% glucose solution.

In vivo : The mean cerebral water content of the normal mice used as controls was 77.6%. in weight. In the case of injured mice, the cerebral water content was found frankly increased to 78.9% and 78.3% of weight in the brain tissue of the injured and the noninjured side, respectively.

The cerebral water content in untreated mice rose to 78.4% in weight after parenteral fluid therapy with 5% glucose 4.5 Gl. 0.09 NaCl, and to

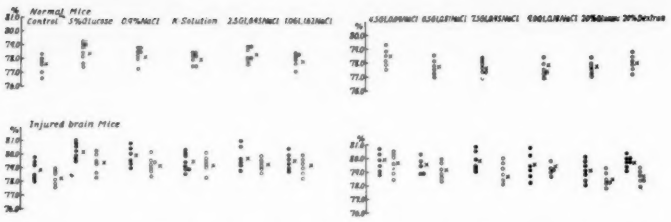

Fig. 2. Change of cerebral water contents giving various parenteral fluids.

In injured brain mice, white circle: non-injured side, black circle: injured side, ×: average.

78.1% after 0.9 NaCl or 2.5 Gl. 0.45 NaCl.

The change of the cerebral water contents was highest with the above two fluids, followed by 20% Dextran, K-solution, 0.5 Gl 0.81 NaCl, and 9.0 Gl. 0.18 NaCl in descending order.

The other kinds of fluids had little influence on the cerebral water content.

The cerebral water content in the injured mice was found more affected in the side injured side than in the intact side.

In the injured side of the brain, the water content increased to the highest value of 80.1% after 5% glucose and the increment decreased after 4.5 Gl. 0.09 NaCl, 0.9% saline, 7.5 Gl. 0.45 NaCl 2.5 Gl. 0.45 NaCl, 20% Dextran, 0.5 Gl. 0.81 NaCl, 9.0 Gl. 0.18 NaCl, K-solution and 1.0 Gl. 1.62 NaCl in the order named.

After 20% glucose, scarcely any change occurred in the water content.

In the intact side, the cerebral water content increased most after 4.5 Gl. 0.09 NaCl, and less after 5% glucose, 9.0 Gl. 0.18 NaCl, 0.9% saline K-solution 2.5 Gl. 0.45 NaCl, 1.0 Gl. 1.62 NaCl, and 0.5 Gl. 0.81 NaCl in descending order, 20% glucose caused little change to the water content of the cerebral tissue.

In the intact half of the brain, 4.5 Gl. 0.09 NaCl had the strongest influence on its water content; the influence decreased in the order of 5% glucose, 9.0 Gl. 0.18 NaCl, 0.9% saline K-solution, 2.5 Gl. 0.45 NaCl, 1.0 Gl. 1.62 NaCl, and 0.5 Gl. 0.81 NaCl, 20% glucose showed no effect on the water content.

B. Change of water distribution in the cerebral tissue and cerebrospinal fluid by various kinds of fluids therapy

Experimental methods and materials

Using 12 rabbits weighing about 2 kg, each 1 cc per kg of weight of

99.87% heavy water plus 40 cc per kg of weight of 5% glucose were injected intravenously 3 cc per minute and 3 cc of blood from the jugular vein, and 0.8 cc of spinal fluid from the cisterna magna were taken 20 minutes and 60 minutes after the injection.

Immediately after the sampling, the rabbits were guillotined without anaesthesia, and the brain were taken out with meticurous care. Then, the consentration of heavy water in the sample of cerebral tissue, spinal fluid and blood were measured.

In this experiment, 0.9% saline, 2.5 Gl. 0.45 NaCl, K-solution, and 1.0 Gl. 1.62 NaCl, as well as 5% glucose were applied for investigation. As a control in this study 1 cc per kg of heavy water was employed.

For measuring the concentration of heavy water, the dropping method[1] was used, as follows: the sample materials were purified by vacuum-distillation and the distillate was dropped from a specially prepared micropipette into the testing tube containing *o*-fluolotoluene and the concentration was determined its dropping speed, at 27.3°C within the temperature error of 0.001°C in a thermostatic chamber. In the measurement, mixture of the cerebral fluid with blood must be avoided.

Results

In the control experiment, the distribution heavy water blood or cerebrospinal fluid was almost of the same value 20 minutes and 60 minutes after the injection of heavy water, and so the transposition of heavy water seems to stop very soon after the injection.

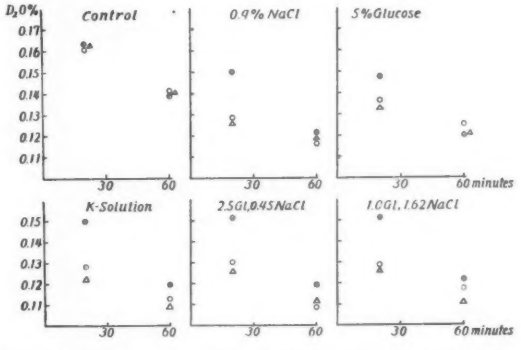

Fig 3. Distribution of D_2O in cerebral matter and C.S.F. giving various parenteral fluids.

Double circle: D_2O in blood, circle: D_2O in C.S.F., triangle: D_2O in cerebral matter.

Various kinds of parenteral fluid were tested in combination with

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heavy water and the concentration of heavy water in the blood was always almost 0.15%, 20 minutes after the injections started.

The concentration of heavy water in the cerebrospinal fluid was 0.13% after 5% glucose and decreased after 2.5 Gl. 0.45 NaCl, 1.0 Gl. 1.62 NaCl, 0.9 saline, in this order.

The lowest value was found after K-solution.

In the cerebral tissues, the change in water distribution showed almost the same tendency as that in the cerebrospinal fluid.

The highest value was found after 5% glucose, the lowest was after K-solution. The concentration of heavy water in the blood 60 minutes after the injection was about 0.12% after any kind of fluid.

The concentration of heavy water in cerebrospinal fluid was found the highest after 5% glucose, followed by 0.9% saline 1.0 Gl. 1.62 NaCl, K-solution, 2.5 Gl. 0.45 NaCl indescending order.

The concentration of heavy water in the cerebral tissue was the highest after 5% glucose and decreased after 0.9% saline, 2.5 Gl. 0.45 NaCl, 1.0 Gl. 1.62 NaCl and K-solution in the order-named.

C. Behavior of intracranial pressure after various kinds of parenteral fluid

Experimental method and materials

Fifteen cats weighing each about 2 kg were intravenously injected with 40 cc per kg of weight of 5% glucose at the rate of 3 cc per kg per minute and the pressure in cisterna magna was observed for a period of 2 hours. Also experimental studies were performed after causing artificial tumor in their brain by means of injection of Penicillin-ointment into the subdural space 48 hours before the parenteral fluid injection.

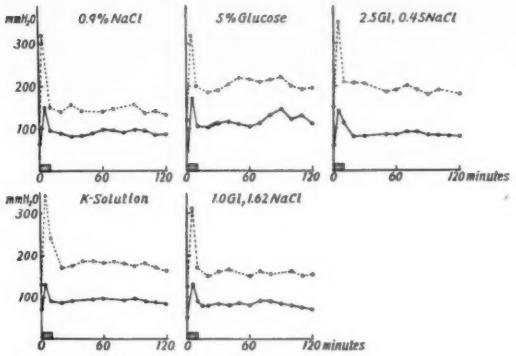
Results

The initial pressure of the cisterna magna of intact cats, was about 50 mm H_2O. As soon as the parenteral fluid was introduced the pressure increased rapidly and the highest value of 125 mm H_2O was found after 5% glucose. In the order of 0.9% saline, 2.5 Gl. 0.45 NaCl, 1.0 Gl. 1.62 NaCl and K-solution, the inerement decreased.

Though the pressure was found to increase after parenteral fluid, the pressure came back to the initial pressure within 10 minutes after the injection started.

Then, the intracranial pressure increased gradually again and reached to the highest point in 40–100 minutes, then decreased gradually to the initial pressure. 96 mm H_2O attained after 5% glucose was the highest level.

In the order of 0.9% saline, K-solution, 2.5 Gl. 0.45 NaCl and 1.0 Gl.



Fib. 4. Change of intracranial pressures giving various parenteral fluids. Dose—30 cc per kg, speed—3 cc per kg per minute. Dotted line: brain tumor cats, line: intact cats.

1.62 NaCl, the rise of the pressure was smaller.

In the case of cats with artificial brain tumor mentioned above, the initial pressure was about 100 to 150 mm H_2O, the change of the intracranial pressure was almost the same as that of intact cats, namely, the first increase of intracranial pressure to 200 mm H_2O was observed within 10 minutes after the parenteral fluid.

The second increase of intracranial pressure was observed remarkably after 5% glucose in the value of 100 mm H_2O.

In the order of 0.9% saline, K-solution, 2.5 Gl. 0.45 NaCl, and 1.0 Gl. 1.62 NaCl, the increment of intracranial pressure was decreased.

Discussion and Summary

In order to find an ideal parenteral fluid which prevents increase of intracranial pressure, namely, causes lesser production of cerebrospinal fluid and prevents cerebral edema, cerebral water contents and the swelling grade of sectioned cerebral tissue after immersion in various kinds of fluids were measured as the first step of this investigation.

In the second step of the investigation, heavy water as a good tracer of water[2)3)4)] was utilized for the study of water distribution in cerebral tissue and cerebrospinal fluid.

The third step of this study was performed in order to elucidate the change of intracranial pressure using the same parenteral fluid.

The swelling grade of sectioned cerebral tissue was found in the lowest degree with 1.0 Gl. 1.62 NaCl in the near isotonic solution and in the order of K-solution, 2.5 Gl. 0.45 NaCl, 7.5 Gl. 0.45 NaCl, 9.0 Gl. 0.18 NaCl, 0.9% saline, 0.5 Gl, 0.81 NaCl, 4.5 Gl, 0.09 NaCl, the grade in-

creased and was the highest with 5% glucose.

The increase of the cerebral water contents of living animals, was of the lowest value after 1.0 Gl. 1.62 NaCl, and in the order of 7.5 Gl. 0.45 NaCl, K-solution, 0.5 Gl. 0.81 NaCl, 9.0 Gl. 0.18 NaCl, and 0.9% saline, the increment rose higher, to the highest after 5% glucose. The study of water distribution in the cerebral tissue, and cerebrospinal fluid showed that the transposition of 5% glucose into cerebrospinal fluid or cerebral tissue was found in the highest grade, and 60 minutes following the start of injection of parenteral fluid was the period of the lowest transposition after K-solution of all the fluids. In the order of 1.0 Gl. 1.62 NaCl, 2.5 Gl. 0.45 NaCl, 0.9% saline and 5% glucose, the transposition into cerebral tissue increased.

The formation of cerebrospinal fluid after parenteral fluid, was the lowest after 2.5 Gl. 0.45 NaCl, and increased in the order of K-solution, 1.0 Gl. 1.62 NaCl, 0.9% saline and 5% glucose.

These experimental results indicated that K-solution caused the lowest shift into the cerebral tissue.

On the intracranial pressure, 1.0 Gl. 1.62 NaCl, caused the minimum increase, and the increment rose in the order of 2.5 Gl. 0.45 NaCl, K-solution, 0.9% saline and 5% glucose. The following conclusion has been obtained that the best parenteral fluid for brain surgery is 1.0 Gl. 1.62 NaCl, K-solution, followed by 2.5 Gl. 0.45 NaCl, 0.9% saline, and 5% glucose in the order named.

In the case injured brain, the effects of the various kinds of fluid were just in the same order as above mention, though the troublesome influences[5] were found more accentuated than in the case with intact brain.

Because water shift into the cerebrospinal fluid after by 1.0 Gl. 1.62 NaCl solution is higher than after K-solution or 2.5 Gl. 0.45 NaCl, it is advisable to apply intraventricular drainage and as the contents of natrium and chloride in 1.0 Gl. 1.62 NaCl are twice as much as those in isotonic solution, careful management of serum electrolyte balance is indispensable.

As a matter of fact, serious patients with brain tumor show highly unbalanced electrolyte contents, so 2.5 Gl. 0.45 NaCl which is prepared as equivalent mixture of 5% glucose and 0.9% saline is recommendable, but single administration of 0.9% saline or 5% glucose should be avoided.

Why did 5% glucose show more noxious effect than the other fluids in our study?

Elliot[6] stated previously that glucose-shift into cerebral tissue is interfered by electrolytes; on the other hand, the shift goes on until equibalent pressure between the cerebral tissue and blood is attained, when no electrolyte is present.

After administration of 5% glucose, Fishman[7] stated that glucose

TABLE I

Summary of the Experimental Results

Numbers show the order of suitability of the various solutions

Methods			Fluids	0.9% Nacl	5% Glucose	2.5% Gl. 0.45% Nacl	0.5% Gl. 0.81% Nacl	4.5% Gl. 0.09% Nacl	K-Solution	10% Gl. 1.62 Nacl	7.5% Gl. 0.95% Nacl	9.0% Gl. 0.81% Nacl
Cerebral water contents	in Vivo	Intact mice		7	9	2	4	8	3	1	5	6
		Injured mice	Injured side	5	9	3	8	7	2	1	4	6
			Non-injured side	6	8	3	9	7	2	1	4	5
	in Vitro	Intact mice		5	6	5	3	7	4	2	1	3
		Injured mice	Injured side	5	6	3	2	6	2	1	4	2
			Non-injured side	2	4	3	2	5	2	2	1	4
Distribution of water contents in cranium		C.S.F.		4	5	1			2	3		
		Cerebral tissue		4	5	3			1	2		
Intracranial pressure		Intact cats		4	5	2			3	1		
		Brain tumor cats		4	5	2			3	1		
Total				4	5	3			2	1		

passes gradually through the blood-brain barrier and shifts into the extracellular fluid and then into the intracellular fluid, finally water follows osmotically and at last brain edema develops.

If so, the experimental fact that the increase of intracranial pressure was lower after 0.9% saline than 5% glucose becomes difficult to explain.

Fishman and Ide[8] answer to this question that water accumulates in the extracellular fluid because the Na-ion are prevented from migrating into the intracellular fluid and does not influence the intracellular fluid, as 5% glucose solution does.

Therefore, it seems to us that the water distribution of brain after 0.9% saline showed lower value in comparison with 5% glucose.

On the other hand, the administration of 2.5 Gl. 0.45 NaCl, showed better results than that of fluids of the above mentioned two groups.

This result may be explained assuming that the glucose-shift can be prevented with elctrolytes and the amount of Na-ions in this solution is

not so large as in 0.9% NaCl and so water accumulates in extracellular fluid in a lower degree.

Conclusion

By measuring the grade of swelling of brain tissue immersed in water solutions of various compounds and varying concentrations and then by measuring the cerebral water content after injection of such solutions as parenteral fluids, the solution causing the least change in the volume of the brain tissue and the cerebral water content was determined. Using heavy water which is known as the best tracer of water, the change in the distribution of intracerebral water under the influence of the different parenteral solutions was next followed up. In the last place, the change in the intracranial pressure, that may be taken to represent the summary of all the changes mentioned above, was measured, to determine which of the tested solutions exerts the least effect on the cranial water content and the production of spinal fluid, and causes the least rise of the intracranial pressure. In summary 1.0 Gl. 1.62 NaCl solution was found to come nearest to the ideal parenteral fluid, followed by K-solution and 2.5 Gl. 0.45 NaCl solution. The worst and the most to be avoided was single administration of 0.9% NaCl or of 5% glucose.

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Studies on Parenteral Fluid Therapy in Neurosurgery

(Second Report)

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(Received for publication, April 14, 1959)

Parenteral fluid therapy in neurosurgery has a distinctive character not to be seen in general surgery and inner medicine.

In brain surgery, prevention of cerebral edema is mandatory, in addition to the correction of defects in alimentary, electrolyte and water metabolism.

Though there has been reported a few papers[1)2)3)4)] concerning this problem, the subjects have been limited to the patients of normotensive intracranial pressure or free of cerebral damage.

After clinical trials on twelve cases, Bakay[5)] stated that 2.5 Gl. 0.45 NaCl was recommended as causing less increase of the intracranial pressure.

Though his study was one of the few good reports on the subject his conclusion was deduced only from measurements of the pressure in subarachnoid space of lumbar region, without measuring the intracranial pressure.

Since the change of intracranial pressure is an important factor for discovering a suitable parenteral fluid for brain surgery, the intracranial pressure was measured with ventricular drainage which has been provided by Dr. Suzuki[6)] in 38 cases of brain surgery.

Because the change of intracranial pressure is an accurate indicator for the rate of injection, amount and kinds of parenteral fluid, measurements of intracranial pressure and electrolyte change under many kinds of parenteral fluid were carried out and finally an adequate parenteral fluid therapy was investigated in this paper.

Experimental method and materials

Thirty-eight cases of cerebral diseases accommodated in Prof. Katsura's surgical clinic were divided into two groups with high and low intracranial pressures, taking the level of 300 mm H_2O as the criterion.

The diseases of these patients were as follows: subtentorial brain tumors, 6 cases; tumors in the pineal region, 5 cases; hypophyseal adenoma and craniopharyngioma, 8 cases; supratentorial meningioma, 3 cases; supratentorial glioma, 3 cases; and other tumors, 13 cases.

The parenteral fluid used in this study were as follows: (1) 0.9 saline, (2) 5% glucose, (3) 2.5 Gl. 0.45 NaCl, (4) K-solution and (5) 1.0 Gl. 1.62 NaCl.

The changes of intracranial pressure and the clinical symptoms were observed for a period from 1 hour before start to 6 hour after end of parenteral fluid administration at the rates of injection of 0.1, 0.2, and 0.3 cc per kg per minutes and in the doses of 10, 20, and 30 cc per kg of body weight of the above mentioned fluids.

The serum electrolyte contents were measured in the samples taken before start and 4 hour after and of parenteral injection.

Chloride and sodium and potassium were measured by means of Schales & Schales' method[7] and flame photometary (Dr. Lange & Co. Ltd. Model V), respectively.

Results

A. Relationship between the intracranial pressure and the rate of parenteral injection

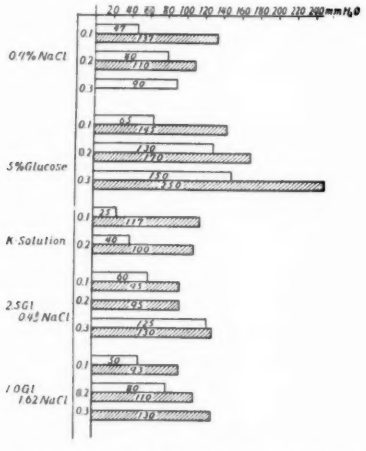

Fig. 1. Relationship between the intracranial pressure and the rate of parenteral injection.

Dose of parenteral fluid: 20 cc per kg, the rate of parenteral injection: 0.1, 0.2, 0.3, cc per kg per minute.

White column: under 300 mmH_2O, shaded column: above 300 mmH_2O.

The increment of intracranial pressure in the low- intracranial pressure group (under 300 mm H_2O) after 20 cc per kg of body weight of 0.9% saline, registered 47 mm, 80 mm, 90 mm H_2O at the speed of injection of 0.1 cc, 0.2 cc and 0.3 cc per kg per minute, respectively. Though the tendency of increase in intracranial pressure after parenteral fluid was almost the same after any other fluid, that after 5% glucose was the highest, namely, 65 mm and 150 mm H_2O at 0.1 cc per kg 0.3 cc per kg per minute respectively.

In the group of high intracranial pressure (above 300 mm H_2O), the intracranial pressure increased more distinctly than in the low-intracranial pressure group, especially in case with 5% glucose, namely, by 145 mm and 250 mm H_2O at the rates of 0.1 cc per kg and 0.3 cc per kg per minute.

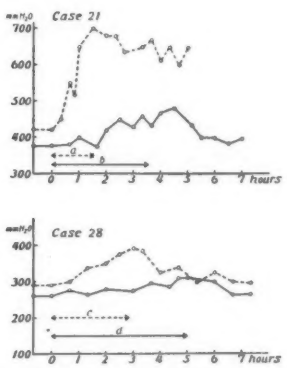

Fig. 2. Relation between the intracranial pressure and the rate of parenteral injection.

Case 21: the kind of parenteral fluid and dose: 2.5% Gl. 0.45% NaCl 500 cc. a: speed of intravenous injection 0.14 cc per kg per minute. b: speed of intravenous injection 0.06 cc per kg per minute.

Case 28: the kind of parenteral fluid and dose: 2.5% Gl. 0.45% NaCl 1500 cc. c: speed of intravenous injection 0.2 cc per kg per minute. d: speed of intravenous injection 0.1 cc per kg per minute.

In the case with 1.0 Gl. 1.62 NaCl at 0.1 cc per kg per minute, the intracranial pressure increased less than with the others. Fig. 2 illustrates this finding as observed in a clinical case.

B. Relationship between the intracranial pressure and doses of parenteral fluid

In the group of under 300 mm H_2O intracranial pressure, the dosis of the parenteral fluid not always affected the increase of intracranial pressure for all kind of the fluids, but K-solution showed the tendency of

Fig. 3

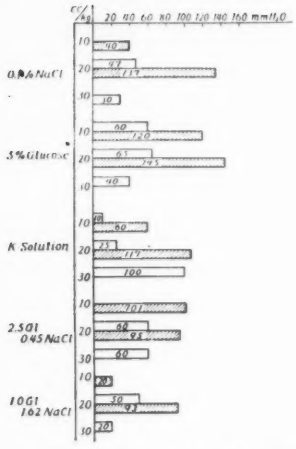

Fig. 4

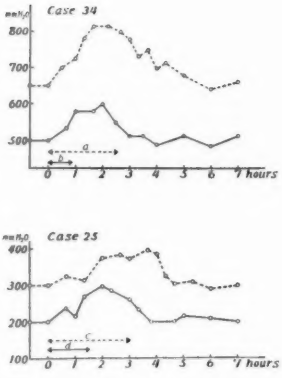

Fig. 3. Relationship between the intracranial pressure and dose of parenteral fluid.

White column: under 300 mmH_2O, shaded column: above 300 mmH_2O.

Fig. 4. Relationship between the intracranial pressure and doses of parenteral fluid.

Case 34: K-solution. a: dose, 30 cc per kg, speed, 0.2 cc per kg per minute. b: dose, 10 cc per kg, speed, 0.2 cc per kg per minute.

Case 25: K-solution. c: dose, 28 cc per kg, speed, 0.15 cc per kg per minute. d: dose, 14 cc per kg, speed, 0.15 cc per kg per minute.

increasing the rise of the pressure with increase in their doses.

In the group of above 300 mm H_2O of intracranial pressure, the higher rise of the intracranial pressure with increase of the dosis of the parenteral fluid was found more remarkable than in the abovementioned group, except instances with 2.5 Gl. 0.45 NaCl.

A clinical case is shown in Fig. 4.

C. Relationship between the intracranial pressure and the kind of parenteral fluid.

The increase of intracranial pressure, independent of the amount or rate of injection of the parenteral fluid was found the highest after 5% glucose following by 0.9% saline, K-solution, 2.5 Gl. 0.45 NaCl, and 1.0 Gl. 1.62 NaCl descending in order.

When the amount and the rate of fluids were 20 cc per kg. and 0.1 cc per kg. per minute respectively, the results were obtained as followed, in the group of under 300 mm H_2O of intracranial pressure, the increase of intracranial pressure was found the lowest after K-solution followed by 0.9% saline, 1.0 Gl. 1.62 NaCl, 2.5 Gl. 0.45 NaCl and 5% glucose in

Fig. 5

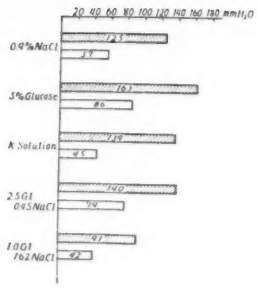

Fig. 6

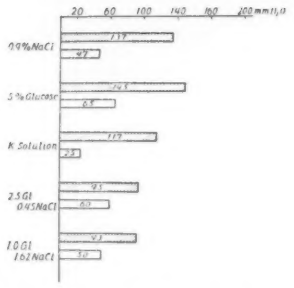

Fig. 5. Relationship between the intracranial pressure and the kind of parenteral fluid.

Average of 38 cases, independent of the amount or rate of injection of parenteral fluid. White column : under 300 mmH_2O, shaded column : above 300 mmH_2O.

Fig. 6. Relationship between the intracranial pressure and the kind of parenteral fluid.

Dose of intravenous injection : 20 cc per kg, speed : 0.1 cc per kg per minute. White column : under 300 mmH_2O, shaded column : above 300 mmH_2O.

ascending order.

In the group of above 300 mm H_2O of intracranial pressure, the increasing of intracranial pressure was in the lowest (93 mm H_2O) after 1.0 Gl. 1.62 NaCl and ascended in the order of 2.5 Gl. 0.45 NaCl, K-solution, 0.9% saline and finally 5% glucose.

Fig. 7 shows a clinical example.

D. Duration of increased intracranial pressure after parenteral fluid.

Studies were performed with 20 cc per kg. of body weight administered at the rate of 0.1 cc per kg. per minute.

In the group of under 300 mm H_2O of intracranial pressure, the duration of increased intracranial pressure was shorter than in the high intracranial pressure group.

The peak and the duration of increased intracranial pressure were the most remarkable after 0.9% saline, followed by 5% glucose, K-solution, 1.0 Gl. 1.62 NaCl, and 2.5 Gl. 0.45 NaCl in descending order.

In the group with high intracranial pressure, the experimental results were almost the same order as in above mentioned group but the duration of increased intracranial pressure after 1.0 Gl. 1.62 NaCl was the shortest, next after 5% glucose, 2.5 Gl. 0.45 NaCl, and K-solution in this order.

The results of experiment in a clinical case were as shown in Fig. 7.

E. Influence of parenteral fluid on serum electrolyte contents

Fig. 7

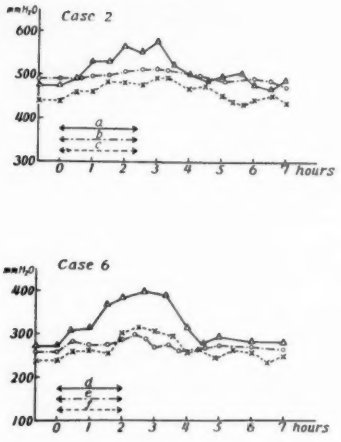

Fig. 8

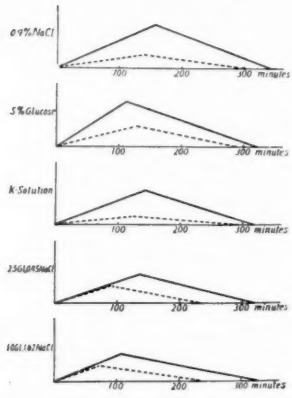

Fig. 7. Relationship between the intracranial pressure and the kind of parenteral fluid.

Case 2 : dose of intravenous injection—20 cc per kg, speed of intravenous injection—0.14 cc per kg per minute. a : 5% glucose, b : K-solution, c : 0.9% NaCl.

Case 6 : dose of intravenous injection—20 cc per kg, speed of intravenous injection—0.2 cc per kg per minute. d : 5% glucose, e : K-solution, f : 0.9% NaCl.

Fig. 8. Duration of increased intracranial pressure after parenteral fluid. Dotted line : under 300 mmH_2O, line : above 300 mmH_2O.

The serum electrolyte contents were measured in 29 cases and supernormal electrolyte concentration was found generally in the cases with high intracranial pressure.

Hypernatraemia, hyperchloraemia and hypopotassaemia were observed after 0.9% saline 4 hours after the end of injection, after 5% glucose, the electrolyte concentration was decreased.

After K-solution, sodium and chloride were decreased and potassium increased.

After 1.0 Gl. 1.62 NaCl the tendency was nearly the same as after 0.9% saline.

There was scarcely change of electrolyte contents after 2.5 Gl. 0.45 NaCl.

Discussion and Summary

All parenteral fluid used in brain surgery show some effects on the intracranial pressure. Since the brain was enclosed anatomically in the

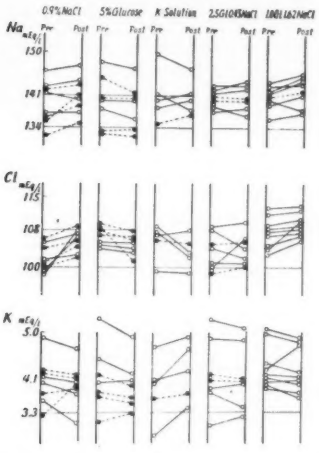

Fig. 9. Change of serum electrolyte contents pre- and post parenteral fluid.
Dotted line: under 300 mmH_2O, line: above 300 mmH_2O.

skull, a rizid bony frame, any increase of the volume of cerebral tissue immediately cause high intracranial pressure. Therefore, an ideal parenteral fluid therapy, which has the feature of preventing intracranial hyperpressure, is needed for brain surgery.

Though intracranical pressure increase more or less after administration of parenteral fluid, its degree is different by the kinds of fluid and the speed, and dosis of injection.

The behavior of the patients with intracranial hypertention or cerebral edema induced by parenteral fluid therapy are quite different from that of other patients.

The subjects were divided into two groups, namely, of those with the intracranial pressure above and below 300 mm H_2O, in this study.

On the kinds of fluids 5% glucose was the worse and 0.9% saline, 2.5 Gl. 0.45 NaCl and K-solution were better in order from point of view of their effect on the intracranial pressure.

1.0 Gl. 1.62 NaCl was best of all the kinds of fluids tested, experimentally and clinically.

In the experiment of raising the rate of injection, no clinical reaction to the worse developed till the rate was raised to 0.45 cc per kg per minute by Dr. Akiu[8] etc. in general surgery.

In spite of this finding injection at the rate of 0.3 cc per kg per minute caused some rise of intracranial pressure in this study.

So, 0.45 cc per kg per minute must be a quite dangerous rate.

In our experiments, the slower the injection, the more the increase in the intracranial pressure was delayed. 0.1 cc per kg per minute is practically recommended as a suitable speed for parenteral fluid injection.

On the dosis of parenteral fluid, Bakay concluded that a large dosis of parenteral fluid had no troublesome effect on the intracranial pressure, but Fishman stated that the intracranial pressure increased in proportion to the dosis of the fluid used.

In our experiments the results obtained were the same as the Bakay's under 300 mm H_2O of intracranial pressure, but Fishman's statement was confirmed in the cases with the intracranial pressure above 300 mm H_2O.

Therefore, more careful parenteral fluid administration should be practised in the case with high intracranial pressure.

Now we face the question of when the intracranial pressure comes to be critical after parenteral fluid injection is started.

The following results will answer this question.

1.0 Gl. 1.62 NaCl caused the shortest-lived increase in intracranial pressure the peak of intracranial hyper pressure appearing 1 hour 50 minutes after the administration of parenteral fluid ended under high intracranial pressure.

Generally 2 hours after parenteral fluid injection, there was the period of highest danger and careful clinical observasion may be necessary for 4 hours on end.

Chen[9] stated that there was many hyper electrolytes in hyper intracranial pressures, in our experiments the results obtained were the same as chen's.

Sodium and chloride, increased after by 0.9% saline or 1.0 Gl. 1.62 NaCl, sodium, chlorine and potassium decreased after 5% glucose, sodium and chlorine decreased and potassium increased after K-solution, there was scarcely any change of electrolyte contents after 2.5 Gl. 0.45 NaCl, and 1.0 Gl. 1.62 NaCl seems to us clinically best of the fluids from point of view of intracranial pressure, and the dosis of 10 to 20 cc per kg at the rate of 0.1 cc per kg per minute is recommended.

If the intracranial pressure is kept at 150 mm H_2O by intraventricular drainage[10], the dosis of the parenteral fluid may be increased much more. But administration of 1.0 Gl. 1.62 NaCl had the tendency of heightening the concentration of electrolytes, so sometimes replacement by K-solution or 2.5 Gl. 0.45 NaCl may be necessary.

Conclusion

By means of accurate measurement of intracranial pressure, an ideal parenteral fluid therapy for brain surgery was investigated.

In our experiments, the slower the injection, the more the increase

Change of intracranial pressure was found in the lowest degree after 1.0 Gl. 1.62 NaCl and next after 2.5 Gl. 0.45 NaCl, K-solution 0.9% saline, 5% glucose in ascending order of the rates of parenteral fluid injection, 0.1 cc per kg per minute was found suitable in our experiments.

The dosis of the fluid had influence intracranial pressure, though the intracranial pressure was not influenced by the dosis of parenteral fluid in the patient of relatively low intracranial pressure.

Patients with postoperative high intracranial pressure were influenced markedly by large dosis of parenteral fluid, so a ventricular drainage should be applied in such conditions.

Observation of the intracranial pressure after parenteral fluid is never to be neglected for a period of 6 hours following the end of injection. The change of serum electrolyte contents was prominent after 1.0 Gl. 1.62 NaCl. Careful attention to the change in the electrolyte content is mandatory in any case.

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Diagnosis of Female Genital Tuherculosis by Means of Hysterosalpingography. Especially on the Clinico-Pathological Figures and Progress of Such Disease

I. Observation of Consecutive 192 Cases of Genital Tuberculosis

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For diagnosis of tuberculosis of the female genitals by findings on hysterosalpingographs (referred to hereunder as HSG), Shinoda[1] and Takahashi[2] in Japan and Rozin[3] in Europe have laid down classifications of the shadows specific to tuberculosis, but few have studied the development of the disease employing HSG, Ekengren & Rydén's report[4] being the only one published recently. The present author will report hereunder the pathological findings and development of tuberculosis obtained in our detailed studies of HSG of tuberculosis cases.

Subjects

192 cases with tuberculosis of the genitals treated in 1950–1953 were examined. For confirmation of diagnosis, cultivation of tubercle bacilli from the genitals (from vaginal contents, menstrual blood, and uterine wash[8]) and histological examination of the endometrium and surgically extracted specimens were always applied. As contrast medium, 40% Molojodol (Lipiodol) was used and the roentgenograms taken 5–10 min. and 24 hours after the introduction of the contrast medium were chiefly used for fundamental study.

Types and shadows examined

The 28 types of shadows listed in Table I were examined.

Results

I. Relative frequency of the shadow. Types in the cases with and without genital tuberculosis

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TABLE I

Comparison of Appearance Rate of HSG Shadows in Tbc. and Non-Tbc. Group

Shadows ↓ Group→	Tbc. confirmed 192 cases	Tbc. not confirmed, 196	Ratio
Uterine shadaws	Out of 240	Out of 199	
Normal uterine cavity	80 (33.3%)	157 (78.8%)	1:2
Small uterus	4 (1.7%)	3 (1.4%)	1:1
Enlarged uterus	8 (3.3%)	5 (2.4%)	1:1
Tiny serration of uterine wall	51 (21.3%)	4 (2.0%)	10:1
Rough serration of uterine wall	10 (4.2%)	9 (4.5%)	1:1
Uterine angiogram	28 (11.7%)	10 (5.0%)	2:1
Dilatation of internal os	5 (2.1%)	2 (1.0%)	2:1
Deformation of uterine cavity	27 (11.3%)	3 (1.4%)	9:1
Atrophy of uterine cavity	22 (9.2%)	5 (2.5%)	4:1
Uterine relief	5 (2.1%)	1 (0.5%)	4:1
Tubal shadows	Out of 619	Out of 426	
Patent tube	31 (5.0%)	195 (45.8%)	1:9
Low-patent tube	7 (1.2%)	53 (12.4%)	1:10
Obstruction at uterine cornu	101 (16.3%)	38 (8.9%)	2:1
Other simple obstruction	18 (2.9%)	45 (10.6%)	1:5
Serration of ampulle	56 (9.0%)	6 (1.4%)	7:1
Relief	103 (16.6%)	21 (4.9%)	3:1
Stricture of ampulle	13 (2.1%)	1 (0.2%)	10:1
Dilatation of ampulle and sactosalpinx	39 (6.3%)	28 (6.6%)	1:1
Round sacculation	60 (9.7%)	12 (2.8%)	4:1
Ragged isthmic shadow	109 (17.6%)	4 (0.9%)	20:1
Dilatation of isthmus	28 (4.3%)	4 (0.9%)	5:1
Dilatation of interstitial part	20 (3.2%)	5 (1.2%)	3:1
Paratubal opaque invasion	14 (2.3%)	5 (1.2%)	2:1
Peritubal relief	6 (1.0%)	1 (0.2%)	5:1
Fimbrial shadow	11 (1.8%)	0 (1.9%)	1:1
Calcification in tube	3 (0.5%)	0 —	—
Pelvic shadows	Out of 192	Out of 196	
Calcification in pelvic cavity	21 (10.9%)	8 (4.0%)	2:1
Adhesion in pelvic cavity	6 (3.1%)	7 (3.5%)	1:1

Table I shows the rough ratios of the frequencies of the shadows appearing in the roentgenograms of 192 cases with confirmed tuberculosis of the genitals and of 196 cases blindly selected from 904 cases without proven genital tuberculosis as controls. It shows that tiny serrations of

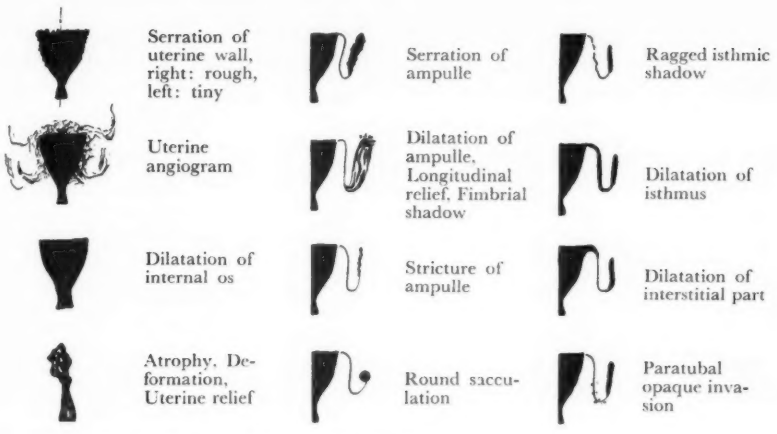

Fig. 1. Schema of Tuberculous Shadows.

the uterine wall, atrophy of the uterus, deformation of the uterine cavity, serration and stricture of the ampulla, serration of the isthmus (ragged-wire-like shadow), round saccultion (cherry-like shadow), tubal relief and various calcification shadows may be deemed as indicative of tuberculosis with considerable reliability and also that uterine angiogram, dilatation of the isthmus, paratubal opaque invasion, and dilatation of the interstitial part are also much more frequent in tuberculous cases.

II. Distribution of combinations of tuberculous shadows

Counting every tuberculous shadow observed as 1 item and two shadows of the same type as 2 items, the distribution of the frequencies of more than one tuberculous shadows in coexistence was as shown in Table II. When the number of tuberculous-type shadows found in one individual case becomes large existence of tuberculosis becomes the more probable, and when 5 or more tuberculous shadows coexist, diagnosis of tuberculosis becomes well-high infullible.

TABLE II

Distribution of Combination Number of "Tbc. Shadows"

Combination No.	0	1	2	3	4	5	6	7	8	9
Tbc. not confirmed, 196 cases	129	31	17	14	5	1	1	0	0	0
Tbc. confirmed, 192 cases	17	21	35	36	24	24	15	10	6	3

III. Classification and correlation of the HSG findings in tuberculosis

As previously reported by the present author[9)10)], the cases of female

genital tuberculosis can be classified into the following three major groups based upon HSG findings: Group I showing perceptible change in neither the uterus nor the fallopian tubes and with the tubal patency intact, Group II with various tubal changes but not marked uterine change and Group III with advanced changes in the uterus too. The salpingographic findings are apparently classifiable into two representative types again: Type A with changes more notable in the ampulla and Type B with changes more notable in the isthmus. The above classifications will be referred to hereunder in studying how they match with the development of genital tuberculosis.

1. *Change in Various Shadows;* As seen in Table III, a peculier change in the ratios of the various shadow types appears when we go over from Group I to Group II. The correlation between the changes in the uterus and the several representative types of tubal changes in combination was as shown in Table IV.

TABLE III

Three Group Classification and Shadow Transition

Tbc. shadow ↓ HSG group→	I (15 cases)	II (131 cases)	III (39 cases)
Patent tube	13 < both 12, one 1	4 < both 2, one 2	0
Low patent tube	2 < both 1, one 1	3 < both 1, one 2	0
Obstruction at uterine cornu	0	45 < both 10, one 35	25 < both 18, one 7
Tbc. tubal shadow Type-A	4 < both 4, one 0	37 < both 27, one 10	1 < both 0, one 1
Tbc. tubal shadow Type-B	0	56 < both 33, one 23	18 < both 10, one 8
Tbc. uterine shadow	3 < slight 3, manifest 0	56 < slight 49, manifest 7	39 < slight 1, manifest 38
Calcification in pelvic cavity	0	11 (8.4%)	10 (25.6%)
Adhesion in pelvic cavity	1	4	0
Culture positive cases	14	98	33
Average of colony (Median)	1.5	2.5	4.0

* 7 cases which are difficult to classfy are excluded

From these findings, we might reasonably conclude that 1) tubal obstruction does not suddenly arise at the uterine cornu but develop from the ampulla to the cornu, 2) the decrease of A type tubal shadows with

TABLE IV

Relation between Uterine and Tubal Changes

Tubal shadow ↓ / Intensity of ut. change (case)→	Normal ut. shadow (80)	Tiny serration of ut. wall (30)	Other tbc. ut. shadow with tiny serration (60)
Serration of ampulle	11.7%	11.4%	6.0%
Tubal relief	25.5%	19.1%	4.5%
Dilatation of ampulle	6.7%	8.4%	2.8%
Ragged isthmic shadow	14.4%	25.7%	28.8%
Round sacculation	11.4%	11.9%	10.6%
Obstruction at ut. cornu	7.4%	8.9%	40.2%
Patent tube	7.7%	2.9%	0
Other tubal shadows	16.2%	11.7%	7.1%

the increase of uterine changes is partly attributable to the progress of tubal obstruction but 3) partly suggests that Type B changes are older in time than Type A changes. The gradual increase in frequency of calcification shadows in the pelvic cavity from Group I to Group III shows that the latter is the oldest in age.

2. *Bacillous discharge in the three groups*

As shown in Table III, the cultivation tests showed nearly always positive results in Group I, because the cases of this group can be seldom detected except by bacillar cultivation. In Group II and III too, positive cases were more frequent but negative cases were not at all rare, and in particular, Group II included about one-fourth of negative cases. Such negative results have been hitherto attributed to defects in cultivation or effects of the menstrual cycle, but we were inclined to infer existence of closed type in genital tuberculosis as in tuberculosis of other sites, and studying the factors leading to such negative bacillus discharge on HSG, arrived at the results in Tables V, VI and VII.

TABLE V

Results of Culture of Vaginal Tbc. Bacilli and HSG Changes of Tubes and Uteri in 2nd Group of HSG (93 Cases)

HSG→	Tbc. involved uteri		Not involved uteri	
Culture ↓	Tubal shadow-A	Tubal shadow-B	Tubal shadow-A	Tubal shadow-B
Positive culture	8	22	21	21
Negative culture	3	4	5	9
Total	11	26	26	30

TABLE VI

Results of Culture of Vaginal Tbc. Bacilli in The View-Point of Menstrual Cycle and Uterine and Tubal Changes in Second Group of HSG (23 Cases)

	With tiny serration of uterine wall			Without tiny serration of uterine wall		
	Tubal shadow type-A	B	A+B	A	B	A+B
Positive culture only at premenstrual and menstrual stadium	3	3	1	2	5	1
Positive culture throughout menstrual cycle	2	2	1	2	0	1

TABLE VII

Results of Culture of Vaginal Tbc. Bacilli and Intensity of Uterine Involvement in Third Group of HSG (39 Cases)

	Positive culture			Negative culture
	Total	Average of colony		
		Over 3	Under 3	
Marked destroyed	15	11	4	3
Not so destroyed	11	6	5	2
Atrophied or deformed	7	1	6	1

As factors affecting the discharge of bacilli in the vagina, we may find 1) the menstrual cycle, as already pointed out in the past, 2) grade of uterine involvement and 3) the findings of the fallopian tubes. As shown in Table V, in Group II of cases almost all free of menstrual anomalies, the discharge of bacilli is seen to be influenced by the presence of anomalies in the uterus, especially, of tiny serrations in the uterine wall, and among the cases free of uterine anomalies, as shown in Table VI, positive cultures are found more frequent among the cases with Type A tubal pictures than those with Type B tubal pictures, both just before menstruation and in the intermenstrual periods. In the Group III of cases with various anomalies in the uterus, as shown in Table VII, the severity of such uterine changes influences both the frequency of positive cases and the quantity of the bacilli in the culture, but in the case the uterine anomalies chiefly consist in its atrophy or deformation, the bacillus discharge is low. From this, we conclude that the Group III may be subdivided into Type A cases with minute serrations or angiograms and Type B cases with uterine atrophy or deformation chiefly appearing on their HSG. Now, as 17 of

the 39 cases in Group III were amenorrheic and the majority of the others hypomenorrheic, it may be presumed that the influence of the menstrual cycle was scarcely felt in the figures in Table VII.

IV. Tiny serrations in the uterine wall

Of all the uterine findings in tuberculosis of female genital organs tiny serrations in the uterine wall are the most fundamental and were found in 25% of our tuberculous cases. Upon closer examination of these serrations, they were found particularly frequent in the fundus of the uterus (Table VIII).

TABLE VIII

Tiny Serration of Uterine Wall

	Fundal serration alone	Fund. serr. intensive, corporal serration slight	Corporal serr. alone	Corp. serr. intensive, fund. serr. slight	Serr. of all ut. wall
With other uterine shadows	2(10.5%)	1 (5.2%)	0	0	16 (84.3%)
Without other uterine shadows	10(23.3%)	12 (27.9%)	6(13.9%)	1 (2.3%)	14 (32.6%)

From this, it may be inferred that the fundamentalmost type of such uterine serrations is those in the fundus uteri, and very probably such fundal serrations are the incipient type of all endometrial serrations. Clinically speaking, we may recommend to select the fundus in diagnostic endometrial curettage as the most promissing point of attack.

SUMMARY

Since methods for successful cultivation of tubercle bacilli from genital organs were established more than a dozen years ago (Shinoda & Iwabuchi[5], 1941; Furuhashi[6], 1947; Halbrecht[7], 1947; Yamaguchi[8], 1952), studies on genital tuberculosis have been encouraged and minute schedules of classification of tuberculous findings based on HSG have been undertaken by Shinoda & Takahashi[2] (1943), Kika, Yamaguchi, *et al.*[9-12] (1951) in Japan and noteworthy reports on the subjects have appeared in Europe too, of Magnusson[14] (1945, 1947), Rozin[3] (1952, 1954), Ekengren & Rydén[4] (1952) and some others[15][16][17].

On the correlation, however, between the different types of tuberculous HSG shadows, their combination and the relation of the findings to discharge of tubercle bacilli we have no report available to date. We have once discussed the pathological pictures and development of genital tuberculosis classifying the cases with the disease into three groups with consideration to the results of cultivation of tubercle bacilli and endometrial

biopsy (Kika, Yamaguchi, *et al.*[9], 1952) and in the present paper, have described the existence of Type A and B among the representative types of tubal findings on HSG, discovered in our closer examination of the HSG of tuberculous cases. From considerations on some relavant findings, we are led to conclude that the Type A findings are younger than the Type B findings and it seems the former develops eventually into the latter. The fundamental type of uterine findings was inferred to consist in the tiny serrations in the uterine walls, which first arise in the fundus near the uterine cornu and gradually spread all over the uterine wall.

These has been no report on the development of genital tuberculosis studied from the angle of HSG findings, except that by Ekengren & Rydén[4] (1950) on the progress of tubal tuberculosis. Their results are in rather good agreement with the ours, but we must state with regret that they failed to study the subject from the bacteriological angle and to review the results in comprehensive discussion with the uterine and the pelvic roentgenographic findings. Our study, we hope, my serve in supplementing some of the shortcomings of their studies.

Conclusions

1. The HSG of 192 cases with genital tuberculosis encountered in these 4 years were examined thoroughly, to discuss the pathological findings and development of the disease.

2. In divergence from the opinion accepted hitherto, we found the diagnostic accuracy obtained by examination of HSG in genital tuberculosis cases is very high, if we take the number of tuberculous findings in coexistence and the combination types of such findings into account.

3. The outstanding combination types of findings in tubal tuberculosis can be classified into the Type A represented by serration in the ampulla and tubal relief and Type B represented by raggedness of the isthmus and round saccultion (cherry-like shadows). Many findings led us to conclude that the Type A temporally precedes Type B.

4. Among the tuberculous uterine findings, tiny serrations in the uterine wall were found the fundamental anomalies. These arise in the fundus and thence gradually spread all over the uterine wall. The uterine anomalies too can be classified in considerations on the results of cultivation of bacilli etc. into Type A with serration and angiograms as the main findings and Type B represented by atrophy and deformation of the uterus. Here, Type A seems to represent the fresher changes than Type B.

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Fig. 2-a. (B-2334)
HSG classification group.
I-A: No pathological change.

Fig. 2-b. (B-2061)
I-B: Tubal patency is still maintained, but slight change of tubes is already seen, especially in the left side.

Fig. 3-a. (B-2185)
II-A: Distinct ampullar findings are visible (Serration, dilatation and relief) but the isthmus and the uterus are normal.

Fig. 3-b. (B-2995)
II-B: Excessive changes of isthmus (ragged isthmic shadow, round sacculation), and serration of uterine wall and dilatation of internal os are also present.

Fig. 4–a. (B-2291)
III–A: Typical uterine angiogram.

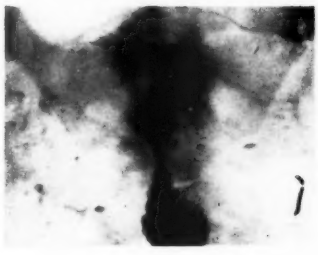

Fig. 4–b. (B-3249)
III–B: Deformation and atrophy of uterus with slight uterine angiogram.

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Diagnosis of Female Genital Tuberculosis by Means of Hysterosalpingography. Especially on the Clinico-Pathological Figures and Progress of Such Disease

II. Observations on 72 Cases Repeatedly Roentgenographed

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In our Report 1, we have classified and analysed the hysterosalpingograms (HSG) of 192 cases of genital tuberculosis. Now that we have had the opportunity of re-examining 72 cases of same disease subjected to repeated roentgenographing in the 15 years past, we will discuss the relation between the progress of the disease and the changes in the HSG in correlation with the results in our Report 1.

The methods of diagnosis and HSG were same as given in Report 1. As contrast medium, 40% Moljodol (Lipiodol) was used throughout.

Results

I. Cases repeatedly photographed without previous treatment

The total HSG taken of the 18 cases of this group numbered 42, the highest number per individual being 4 and the longest period of observation 11 years. The main transition of the shadows of the fallopian tubes, the uterus, and the pelvic cavity are shown in Figs. 1, 4 and 5.

1. In the uterus, the normal uterine contour was seen to show tiny fundal serration, then tiny serration and uterine angiogram at least (Fig. 1-1, 2).

2. The changes in the tubes were rather complicated, but in general, the change passed from a normal shadow to pathological shadow in the ampulle (as in tubal shadow Type A described in Report 1) seems to develop to anomaly in the isthmus (Type B). The blockage proceeding from the fimbriated end to the ampulle, to the isthmus and thence to the uterine cornu. This progress is in total agreement with what shown in Table IV in Report 1. The changes of finding of individual tubes are very

complicated and hard to interpret, owing to the multiplication of photographing conditions, but generally speaking, we may assume the following series of changes: *At the ampulla:* dilatation→round sacculation (Fig. 1–6); round sacculation→round sacculation accompanying speckled relief (7); longitudinal relief→the same with dilatation (8); normal ampulle →stricture (3), round sacculation (4) or longitudinal relief (5). *At the isthmus:* ragged isthmic shadow→isthmic dilatation (9); isthmic dilatation→ragged isthmus (9); round sacculation→paratubal opaque invasion (10); normal isthmus → raggedness and/or dilatation of isthmus (11, 12). The HSG findings in oviducts are labelled "unchanged" in the largest number of cases in this untreated group, showing that the disease takes a very chronic process if left without treatment.

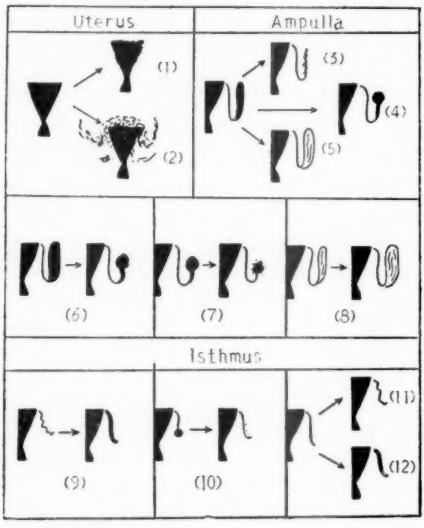


Fig. 1. Shadow transition in untreated group.

The number of years required for the above changes in the tubes, the uterus and pelvic cavity to appear on HSG have been summarized in Table I.

On the viewpoint of required years to develop to each of three groups of HSG, it may be tabulated as shown in Table II. That is, the changes from HSG group I to II takes more than 10 years and from II to III more than 3 years. The years from the first anamnesis of tuberculosis to the first examination and the groups of HSG at the first examination showed results approximating the data given in Fig. 2.

TABLE I

Years Required for Representative Symptoms to Change. (Number of Photographed Shadows)

First HSG		Transisted shadow	Elapsed years				
			~1	~3	~5	~10	11~
Patent tube		Tubal seadow A		2			
Tubal shadow	A	A		2			
		B		1			
		A+B	1			2	
		Corn. blockage				1	1
	B	B	1		1		
		Born. blockage		1			
	A+B	B		2	1	1	
		A+B	2	1		1	
	Cornual blockage	Unchanged		2			
Uterine shadow	Normal	Normal		3	1		
		Tiny serration with angiogram	1			1	1
	Tiny serration	Improved		4		1	1
		Unchanged	3	1		3	
		Angiogram and/or others		1	2		
	Angiogram	Unchanged	1	1			
Pelvic shadow	Negative	Adhesion	1				
		Calcification				1	
	Adhesion	Calcification				1	
		Increased		1			
	Calcification	Unchanged	2	1		1	

* Tubal shadow A: With more anomalies in ampullar part
Tubal shadow B: With more anomalies in isthmic part

** When a cases was photographed thrice or oftener, the results are given in repetition.

II. Cases repeatedly roentgenographed following chemotherapy

This group of cases comprises 54 individuals, and 129 HSG were taken of them. By individuals, the highest number of HSG was 5 and the longest period of observation 4 years, and the majority being kept under observation for 1–2 years. The antibiotics and chemotherapeutics were streptomycin (intramuscular and intracervical injection, or utero-tubal jelly-instillation), PAS (per os), INAH (per os or by jelly-instillation) and Thiosemicarbazone (per os or by jelly-instillation), used singly or in combination.

TABLE II

Transition of HSG Classification Group. Relation between Elapsed Years

HSG group ↓ Elapsed years→	~1	~3	~5	~10	11~
I → I		1			
I → II	1	1		1	1
I → III				1	1
II → II	4	7	1	2	
II → III		2			
III → III		2			

* Figure shows number of photo.

** When a case was photographed thrice or oftener, the results are given in repetition

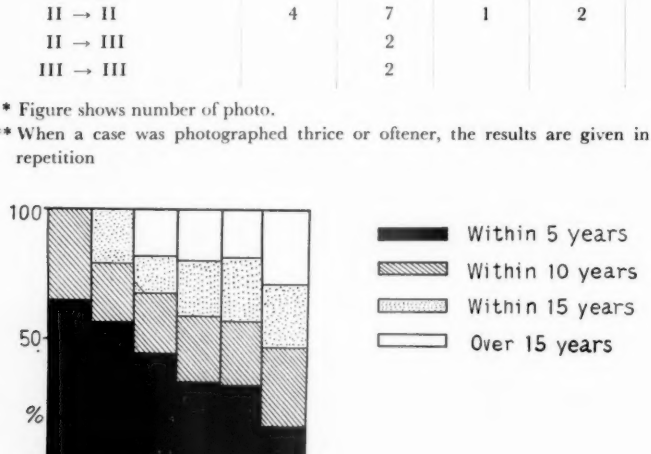


Fig. 2. Comparison of elapsed years from the first history (192 cases of report 1, proportion of each 5 years).

1. *The ampulle* (Figs. 3, and 5): The findings most liable to change were dilatation, serration and stricture, but longitudinal relief and round sacculation showed little change. The changes usually progressed as follows: dilatation of ampulle → round sacculation (Fig. 3–1); serration of ampulle → longitudinal relief (2), stricture (3), paratubal opaque invasion (4); round sacculation → speckled relief (6), stricture (5), paratubal opaque invasion (5) or dilatation. On the findings after the completed course of chemotherapy, " unchanged " images were most predominant in all parts. As relapse of once alleviated symptoms may be enumerated that the ampullar dilatation, of longitudinal relief and of ampullar serration, and as changes to other shadows: ampullar stricture → paratubal opaque invasion (7) and rosary-like sacculation (8); ampullar dilatation → sactosalpinx-like large round sacculation (9) and stricutre (10), may be

pointed out.

In short, we may conclude that dilatation, serration and stricture of ampullar part are reversible changes, apt to alleviate but easy to recur upon suspension of treatment, while longitudinal relief and ampullar round sacculation are comparative fixed changes, little affected by chemotherapy.

2. *The isthmus* (Fig. 3): The shadow most liable to improvement is ragged isthmic shadow, but the other findings are little affected. This is a point of wide difference between the ampullar and isthmic findings. This means perhaps that while an anomaly in the shadow of the isthmus indicates an older state of disease in the tubes than the anomalies in the ampulla, viewed from histological as well as HSG standpoints (Kan[6], 1957). Outstanding changes of HSG may be listed: ragged isthmus → dilatation of isthmus (Fig. 3–11) and paratubal opque invasion (12); dilatation of isthmus → ragged isthmus (13). On the other hand, the findings after the completion of the therapeutic course were mostly " un changed ", but some cases of the changes, dilatation of isthmus → paratubal opaque invasion (14); ragged isthmus → paratubal opaque invasion (12) or dilatation of isthmus (11); normal isthmus → dilatation of isthmus, were also found.

In short, the anomalies in the isthmus show comparatively little changes in HSG indicating little reaction to chemotherapy.

3. *The uterus;* The change most susceptible to improvement is here tiny serration of the uterine wall, and indeed, this anomaly is most sensitive to change of all anomalies found in HSG. The uterine angiograms are also rather improvable, seemingly due to the very much better reparation to which the endometrium is liable than the tubes. On the other hand, the uterine atrophy and deformation are hard to improve. In the cases of post-treatment observation, no marked change is found. This seems to prove that uterine tuberculosis either reacts readily to chemotherapy and becomes fixed in contrast to tubal tuberculosis so that its HSG is also not so changeable.

4. *The pelvic cavity;* The shadow of adhesion shows improvement but the shadow of calcification naturally undergoes no change at all.

Comment

On the results of the above and of our Report 1, we will give some discussion on the clinical features of this disease.

I. Mutual relation of various anomalous shadows

1. There are two ways for shadows of ampullar round sacculation to develop, as follows; i) because of partial stricture (a stricture of ampullar lumen caused by an incrassation of mucosal fold), the ampulle appears in the HSG constricted and as a shadow of atypical round sacculation in

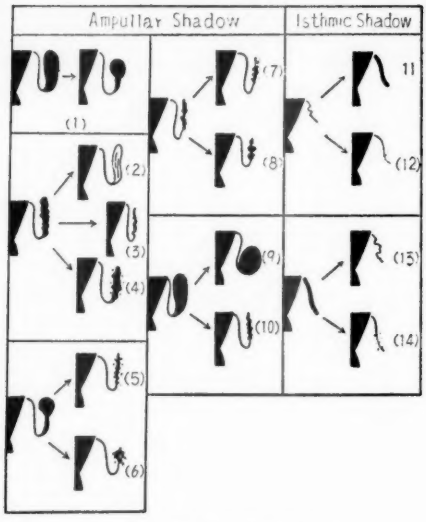
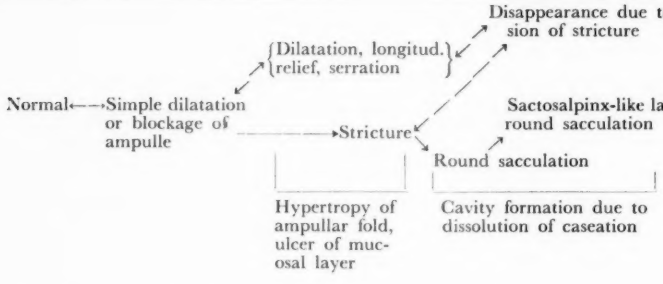


Fig. 3. Shadow transition after chemotherapy.

a rosary arrangement (Fig. 7–a), and ii) occasioned by the excavation of strong caseation in the ampullar lumen (Figs. 8–a, b). When the latter degeneration advances and spreads over the entire ampullar lumen, the shadow on HSG should develop into a large round sactosalpinx with serrated wall (Fig. 8–c).

TABLE III

Development of Ampullar Shadow

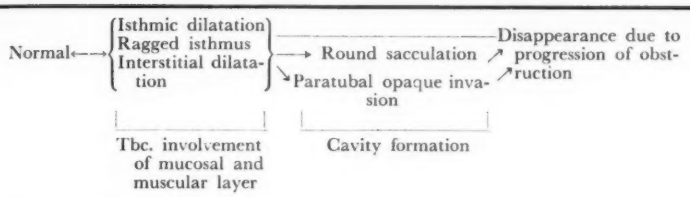

Disappearance due to progression of stricture
Dilatation, longitud. relief, serration
Normal
Simple dilatation or blockage of ampulle
Stricture
Sactosalpinx-like large round sacculation
Round sacculation
Hypertropy of ampullar fold, ulcer of mucosal layer
Cavity formation due to dissolution of caseation

2. For the development of the isthmic shadows, we may assume the pattern shown in Table IV.

A shadow of round sacculation in the isthmus often accompanies that

TABLE IV

Development of Isthmic Shadow

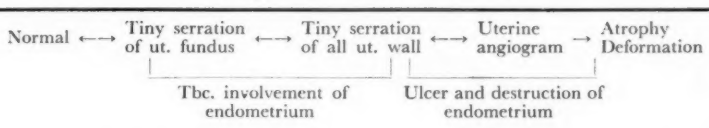


of paratubal opaque invasion and changes into the latter during untreated progress or after chemotherapy. This shows that the shadow of paratubal opaque invasion is sometimes developed from that of isthmic round sacculation (Fig. 9). It is known that the isthmic round sacculation is sometimes induced by the cavitation of " salpingitis isthmica nodosa " (Kan[1], 1957), hence it must be born in mind that the isthmic paratubal opaque invasion indicates the prodrome of cavitation.

3. A schematic representation of the uterine shadows is shown in Table V.

TABLE V

Development of Uterine Shadow



Among the changes, the uterine angiograms are known to be due to an advanced degeneration, ulcerous or caseous, of the endometrium (Miyano[7], 1957). On the other hand, the shadow of atrophy and deformation may be rather taken as signs of ceased and scarred state of this disease.

II. Development of genital tuberculosis

The cases of female genital tuberculosis can be divided into three groups as mentioned in our Report 1. We would further divide those three groups into two subgroups each, as shown in Table VI, in consideration of the results of experiments described in our first and this reports.

The relative progress of the pathological changes may be something as summarized in Table VII.

CONCLUSIONS

1. The author made investigations on 18 cases of repeatedly roentgenographed untreated and 54 cases of similarly photographed cases sub-

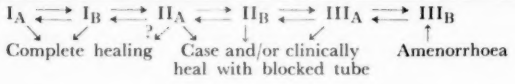
TABLE VI

HSG Classification of Genital Tuberculosis

I	A. . .	No finding in the uterine, tubal and pelvic shadows, that is normal HSG
	B. . .	Some changes are visible, but slight. Tubal patency is still good
II	A. . .	Significant ampullar shadows are presented, but isthmic involvement is still slight
	B. . .	Marked isthmic involvement is seen, but ampullar shadows are not marked or invisible due to progression of tubal blockage
III	A. . .	Marked uterine involvement is visible, for example, tiny serration of uterine wall with angiogram, typical angiogrom with other uterine shadows
	B. . .	Atrophy, and/or deformation of uterine contour

TABLE VII

Development of HSG Group of Genital Tuberculosis



jected to chemotherapy, 72 cases in total, comparing the results of Report 1, having discussed the clinico-pathological features, progress and the mutual relations of the different observed shadows.

2. The cases of female genital tuberculosis can be classified by HSG into three groups of I, II and II, which may be subdivided into two groups each of A and B.

3. The disease may be assumed to progress thus: $I_A \rightleftarrows I_B \rightleftarrows II_A \rightleftarrows II_B \rightleftarrows III_A \rightarrow III_B$.

4. The cases that may be expected to recover with preserved patency of the fallopian tubes are those belonging to IA and IB, but reopening of the tubes is seemed possible in IIA cases too, but not in more advanced cases.

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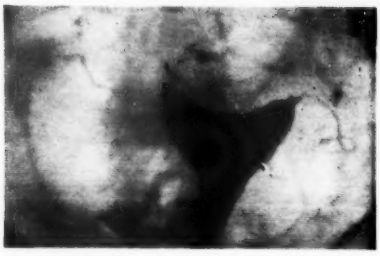


Fig. 4–a. Case B-886
The uterine wall is serrated, and both tubes are seemingly patent.

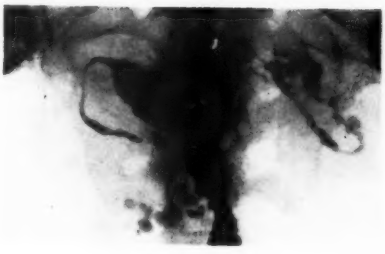


Fig. 4–b. Same patient, 10 years later.
The uterine wall is considerably serrated, both isthmi are dilated and ragged, and the left ampulla is markedly constricted (stricture) with paratubal opaque invasion. The tip of the right tube is sacculated, and the left is speckled. Moljodol residue or calcification is in the right side.

Fig. 5–a. Case B-1639
The uterine wall is almost normal but uterine angiogram is seen in some degree. The right isthmus is slightly ragged. The right ampulla is constricted and shows rosary-like round sacculation.

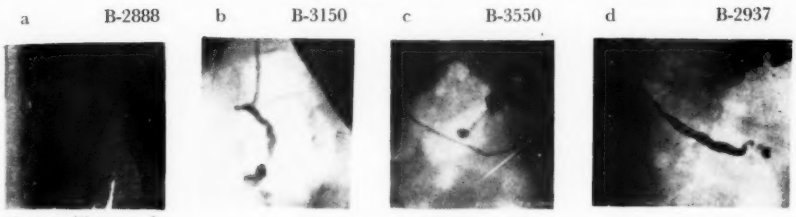
Fig. 5–b. Same patient, 3 years later.
The uterine wall is almost normal. The right ampulla is constricted with speckled relief (round sacculation?). The left isthmus is ragged. In the mid-portion of the isthmus, typical round sacculation is present.

Fig. 6-a. Case B-3411
Before chemotherapy. The uterine wall is serrated. Both ampullae are serrated and dilated, and distinct longitudinal relief is recognized.

Fig. 6-b. Same patient during chemotherapy (INAH, PAS)
Tiny serration of uterine wall has disappeared. Dilatation, serration and longitudinal relief of both ampullae have also disappeared. Right ampulle is slightly constricted (ampullar stricture).

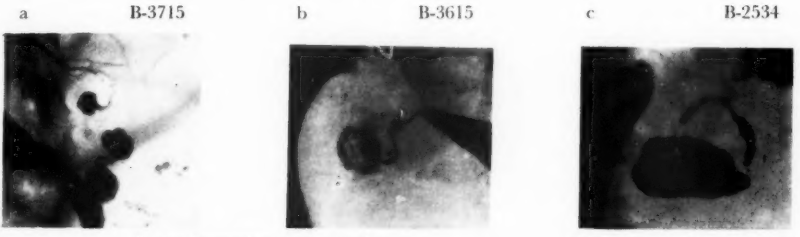
Fig. 6-c. Same patient 3 months after chemothrapy
Ampullar changes begin to recur.

Fig. 6-d. Same patient 6 months after chemotherapy
Quite same to HSG before chemotherapy. Culture of tubercle bacilli have been positive again.



Rosary-like round sacculation due to partial stricture.

Fig. 7. Progress of stricture in the ampulle.



Cavity formation is developing

Cavity formation completed

Fig. 8. Development of round sacculation of the ampulle.

(B-3247)

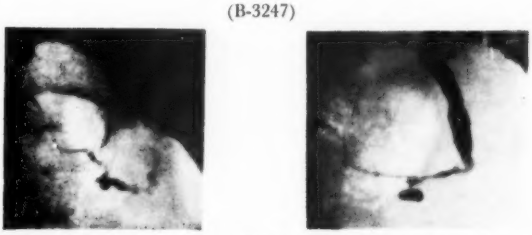
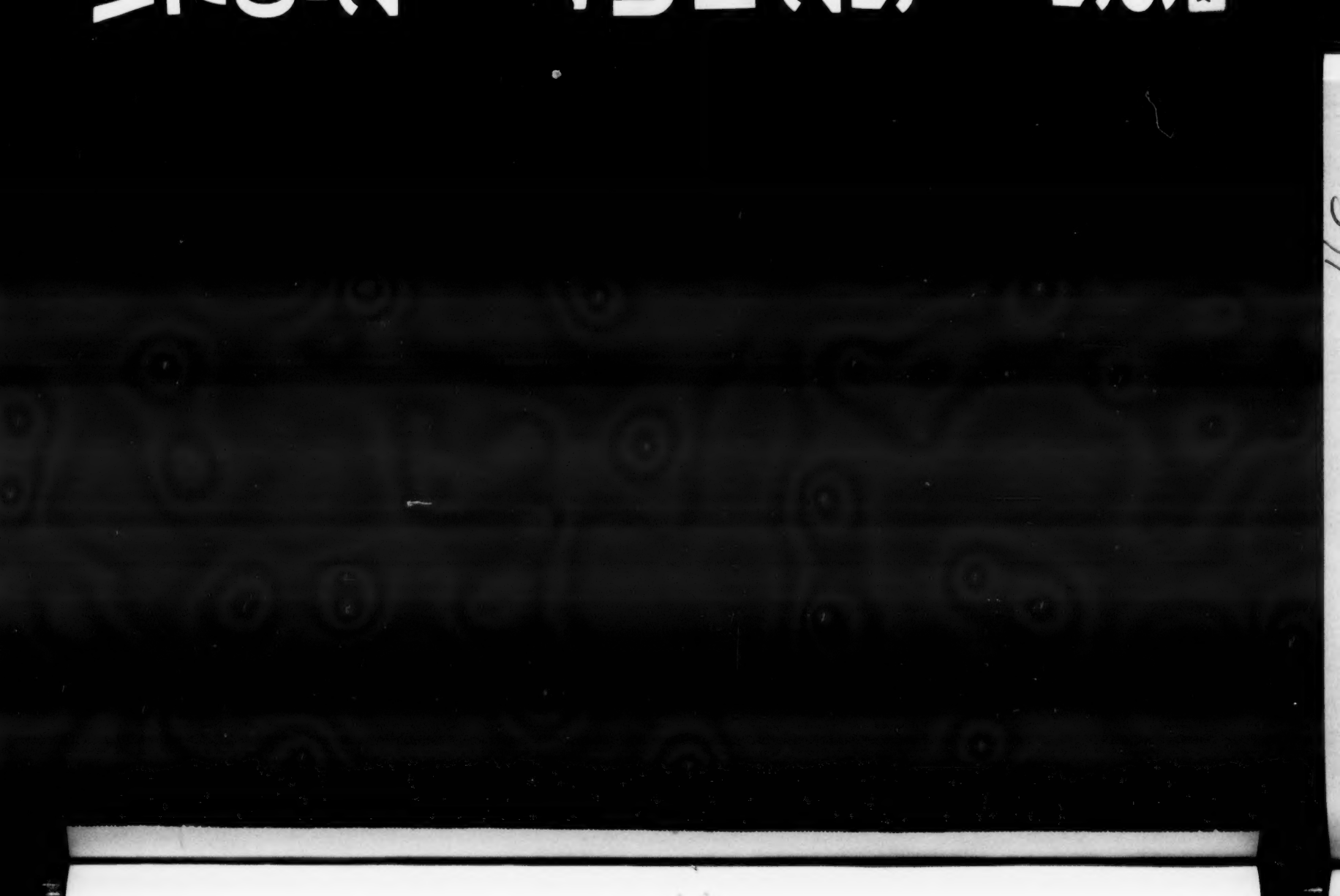



Fig. 9. The Relation of paratubal opaque invasion and round sacculation of isthmus. (Same Patient. Right, before chemotherapy; left after chemotherapy).